[redacted] Summary of Current Program, 7/1/63,

and Preliminary Report of Progress

for 7/1/62 to 6/30/63

ANIMAL DISEASE AND PARASITE

RESEARCH DIVISION

of the

AGRICULTURAL RESEARCH SERVICE

UNITED STATES DEPARTMENT OF AGRICULTURE

This progress report of U.S.D.A. and cooperative research is primarily a tool for use of scientists and administrators in program coordination, development and evaluation; and for use of advisory committees in program review and development of recommendations for future research programs.

The summaries of progress on U.S.D.A. and cooperative research include some tentative results that have not been tested sufficiently to justify general release. Such findings, when adequately confirmed will be released promptly through established channels. Because of this, the report is not intended for publication and should not be referred to in literature citations. Copies are distributed only to members of Department staff, advisory committee members and others having a special interest in the development of public agricultural research programs.

This report also includes a list of publications reporting results of U.S.D.A. and cooperative research issued between July 1, 1962 and June 30, 1963. Current agricultural research findings are also published in the monthly U.S.D.A. publication, Agricultural Research. This progress report was compiled in the Animal Disease and Parasite Research Division, Agricultural Research Service, U. S. Department of Agriculture, Beltsville, Maryland.

UNITED STATES DEPARTMENT OF AGRICULTURE
Washington, D. C.
July 1, 1963

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INTRODUCTION

Animal disease and parasite research, as used in this report, is concerned with infectious, non-infectious, and parasitic diseases of cattle, swine, sheep, goats, horses, poultry, and fur-bearing animals. It involves fundamental investigations of causes and effects of diseases as they affect economic farm and ranch production.

The Animal Disease and Parasite Research Division has 38 scientists at the Beltsville Parasitological Laboratory, the National Animal Disease Laboratory, Ames, Iowa, has 92.5 budgeted professional positions, and the Plum Island Animal Disease Laboratory, New York, has 42.5 budgeted positions, in the current fiscal year. The rest of the Division's total complement of 230 budgeted scientific positions, or 58, is distributed among small groups at 11 smaller, specialized field stations, which are located at Auburn, Alabama; Fontana,California; Denver, Colorado; Live Oak, Florida, Athens and Tifton, Georgia; Albuquerque and State College, New Mexico; Kerrville, Texas; Logan, Utah; Pullman, Washington; and at foreign missions at Amsterdam, Holland, and Kenya, East Africa.

Federally supported research under 54 contracts or cooperative agreements with State stations is equivalent to 15.8 professional man-years.

Man's needs and wants for animal products, and public health and welfare, are inseparably connected with healthy and economically profitable livestock production. In some parts of the world, animal diseases exist that jeopardize human existence. Fortunately, many animal diseases do not exist in the United States, however, they pose a continuing threat to livestock production in this country. Disease in animals inevitably affects human welfare, either because people become infected or they are deprived of needed food and other products derived from animals. Diseases in the lower animals do not differ essentially from those in man, and frequently infections are interchangeable among them. More than half of the total farm income in the United States is derived from livestock and livestock products. Exact calculation of the extent of reduction of income by animal diseases is difficult. It has been estimated, however, that as many as 1/10 of the domestic animal population is lost each year through incursions by disease in one form or another. Over-all losses due to disease have been estimated to amount to at least $2 billion annually. Such losses must be reduced to minimums if production of meat, milk, leather, wool, and other essential animal products is to keep up with progressively increasing demands.

The Animal Disease and Parasite Research Division and its predecessors have made many significant contributions to the welfare of the livestock industry and to mankind in general. The discovery in 1893 that Texas fever of cattle was transmitted by ticks was one of the great chapters in biomedical history.

It was the first proof that arthropods could transmit infectious diseases and opened the door to research on malaria, yellow fever, typhus, Rift Valley fever and other insect-borne diseases of man and animals. In 1904 the discovery that hog cholera was caused by a virus led to the future development of immunizing procedures against this devastating disease. In 1930 Strain 19 vaccine was developed to assist in the control of brucellosis. In 1960 a diagnostic test was developed which differentiates between hog cholera and African swine fever. More recently our scientists have developed fundamental knowledge on the structure and heat resistance of foot-and-mouth disease virus. It was also found that foot-and-mouth disease could be transmitted through infected semen. A new diagnostic procedure for hog cholera, using fluorescent antibody techniques, has been developed. However, new problems seem to arise as fast as old ones are solved. We have only scratched the surface in our efforts to develop knowledge that will reduce losses from animal diseases and parasites.

Expansion of basic research is needed on host-parasite relationships, the relation of chemistry and enzyme systems of causative agents to their pathogenicity, the nature and resistance to disease and methods of breeding resistant animals. Increased studies should be made of growth requirements of disease-causing agents, methods of destroying such agents, methods of diagnosis and treatment of disease, and the detection of carriers of disease. Research at the present level will not be adequate to provide in the '70s the same standard of living enjoyed today. Since the results of research conducted today will not be available, as a general rule, until at least 10 years after the work is started, increases in research are necessary.

With the projected supplementation of facilities at the Beltsville Parasitological Laboratory, the National Animal Disease Laboratory, the Plum Island Animal Disease Laboratory, and carefully selected subsidiary field stations, it is now possible for the first time in many years to foresee a comprehensive program of research that is reasonably commensurate with national needs. The basic aim of research in all fields in the United States is protection and satisfaction of human needs. Man's problems are closely related to disease in animals, and expenditures for research on animals should accordingly be more reasonably commensurate.

AREA NO. 1 - INFECTIOUS AND NON-INFECTIOUS DISEASES OF CATTLE

Problem. Losses from infectious and non-infectious diseases of cattle, other than those due to parasites, are estimated at approximately $600 million annually. These losses materially increase costs of production and conversely decrease profits. In turn, they contribute to the cost of every purchase of meat, milk, and other cattle products to the consumer. Some of these diseases are transmissible to man. Determination and definition of the causes of cattle diseases, explorations for efficient methods of diagnosis, prevention, control, and when feasible, eradication, are the purposes of the research program.

USDA PROGRAM

The Department has a continuing long-term program involving biochemists, microbiologists, pathologists, and veterinarians engaged in both basic studies and the application of known principles to the solution of infectious and non-infectious diseases of cattle. Research is being conducted on the diseases at the designated locations.

The Federal scientific effort devoted to research in this area totals 57.7 professional man-years. This effort is divided among sub-headings as follows:

Brucellosis, 2.3 at the National Animal Disease Laboratory, Ames, Iowa, and under cooperative agreements with the Universities of Maryland, Minnesota, and Wisconsin.

Vibriosis, 5.1 at the National Animal Disease Laboratory, Ames, Iowa, and under cooperative agreement with the New York State Veterinary College at Ithaca.

Tuberculosis, 6.6 at the National Animal Disease Laboratory, Ames, Iowa, and through two contracts with the Michigan State University, East Lansing.

Mucosal-Respiratory Disease-Complex, 5.1 at the National Animal Disease Laboratory, Ames, Iowa, and under cooperative agreements with the Indiana (Lafayette) and Iowa (Ames) Experiment Stations, and the Colorado State University (Fort Collins).

Mastitis, 6.2 at the National Animal Disease Laboratory, Ames, Iowa, and under a cooperative agreement with the University of California, Davis.

Respiratory Disease (Shipping Fever), 5.0 at the National Animal Disease Laboratory, Ames, Iowa.

Leptospirosis, 6.0 at the National Animal Disease Laboratory, Ames, Iowa.

Infertility in Cattle, other than vibriosis and trichomoniasis, 3.0 at the National Animal Disease Laboratory, Ames, Iowa.

Epizootic Bovine Abortion, 3.4 at the National Animal Disease Laboratory, Ames, Iowa, and under a cooperative agreement with the University of California, Davis.

Foot Rot, 4.0 at the National Animal Disease Laboratory, Ames, Iowa.

Paratuberculosis (Johne's Disease), 5.0 at the National Animal Disease Laboratory, Ames, Iowa.

Keratitis (Pink Eye), 2.0 at the National Animal Disease Laboratory, Ames, Iowa.

REPORT OF PROGRESS FOR USDA AND COOPERATIVE PROGRAMS

A. Brucellosis

Research workers at the National Animal Disease Laboratory (NADL), Ames, Iowa, reported the pathology of 2 bulls, naturally infected with Brucella abortus, was studied for 5 and 2 years, respectively. Serologic, bacteriologic and histopathologic examinations were correlated with the clinical signs of the disease. Seroagglutinin and semen plasma agglutinin titers persisted at diagnostic levels throughout the study, and Br. abortus was consistently isolated from semen of both bulls. At necropsy, Br. abortus was isolated from the testes, epididymides, seminal vesicles, and the ampullae of the ductus deferens. Pathologic changes were observed throughout the genital tract. Granulomas, including sperm granulomas, were found in the epididymis of one bull.

Modern techniques for processing and distributing semen from such bulls create a situation, wherein thousands of cows may become infected with brucellosis. Semen plasma agglutination tests, seroagglutination tests, and bacteriologic examination of semen offer the best means of detecting an early or clinically inapparent infection.

Recent research has been directed toward differentiation of Brucella agglutinins in bovine serums on the basis of their heat stability at 65 C for 15 minutes. By this criterion a heat labile and a heat stable seroagglutinin have been demonstrated. Ultracentrifugation revealed that the heat labile seroagglutinin had a higher molecular weight than the heat stable seroagglutinin. The heat labile agglutinin was found in the serums of cattle known to be brucellosis-free; in the serums of calves after vaccination with Brucella abortus Strain 19; and in serums of heifers with persistent postvaccinal seroagglutinin titers. It was also found in the serums of cattle recently exposed to virulent Brucella abortus and in serums of cattle that became infected. The heat stable agglutinin was found only in the serums of cattle that were infected or exposed to Brucella abortus. The heat labile

seroagglutinins were predominant at the onset of the disease, whereas the heat stable seroagglutinins became predominant as infection progressed. Heat stable Brucella seroagglutinins were an indication of exposure to Brucella abortus, whereas the significance of the heat labile seroagglutinins was not as readily apparent.

Acidified Plate test antigens at pH 4.0, 3.6, 3.4, 3.2, and 3.0 were evaluated in supplementary tests to clarify the status of "suspect" cattle to standard seroagglutination tube and plate tests for bovine brucellosis.

The serologic response of 57 vaccinated and 22 nonvaccinated animals was studied after conjunctival exposure in midgestation to about 7×10^5 cells of virulent Brucella abortus Strain 2308. The brucellosis status of each animal at the termination of pregnancy was determined by bacteriologic examination of blood, milk, uterine contents and fetal organs.

The acidified plate antigens were compared for their inhibitory effect on serologic reactions of infected and noninfected cattle.

Inhibition of the suspect serologic reactions was directly dependent upon the magnitude of the original standard seroagglutination tube or plate test titer and the pH of the antigen. Serologic reactions in the lower suspect titer range were more readily inhibited. The inhibitory effect increased gradually as the final pH of the antigen-serum mixture decreased.

Since low level seroagglutination reactions involving specific Brucella agglutinins were frequently inhibited by the antigens, such antigens have little value in supplemental tests to determine the brucellosis status of "suspects" in an infected or problem herd. (NADL)

The University of Minnesota, under a cooperative agreement with the USDA, continued the studies of macroglobulins of milk and serum, but the emphasis was on developing methods of application of basic findings. Studies during the past year have been concerned with evaluating a new test procedure to detect macroglobulins for Brucella in cattle serums and milk, studying the occurrence and distribution of these agglutinins in a certified county, and studying their appearance and persistence in experimentally infected swine. (Minnesota)

The University of Wisconsin, under a cooperative agreement with the USDA, initiated a systematic study of the complement-fixation test for use as a diagnostic procedure in the research on brucellosis. A standardized method for the test has been developed. Preliminary data indicate that the test is a useful supplemental test for subjects in problem herds. It permits differentiation of infected vaccinated heifers from non-infected vaccinated heifers with persistent agglutinin titers. Other data comparing the CF with other supplemental tests are being analyzed.

In the immunochemical determinations, phenol extracts from Br. abortus have been compared with those previously obtained by some disruption of cells. These have been studied by serological and biological methods. (Wisconsin) (ADP a1-3(Rev.)

B. Vibriosis

The National Animal Disease Laboratory (NADL) workers reported their investigations were directed toward analysis of the cellular changes in the uterine endometrium due to V. fetus infection. Twelve virgin heifers were infected by natural service to a V. fetus-infected bull. At intervals of 8 to 122 days after initial exposure, the heifers were necropsied and uterine sections obtained for histopathological studies. Five unbred heifers and 7 heifers bred to a noninfected bull, were necropsied at intervals of 0 to 39 days after estrus or breeding and uterine sections were obtained for comparative studies as controls.

All heifers bred to the infected bull became infected at first service and microscopic studies of uterine sections revealed neutrophilic and lymphocytic periglandular infiltration of the endometrium that was not observed in noninfected heifers. The presence of a moderate inflammatory process suggested the underlying cause for lack of conception and subsequent pregnancy. Histologic differentiation was not observed in the cervicovaginal areas, cervices, or ovaries of any heifers. Only 2 of 12 infected heifers had evidence of pregnancy when euthanized compared with 6 pregnant of the 7 noninfected heifers. (NADL)

The New York State Veterinary College, Ithaca, under a cooperative agreement with the USDA, continued research investigations on diagnostic procedures for vibriosis. One of the principal difficulties encountered in the control of vibriosis is the diagnosis of the asymptomatic carrier state in the bull. Cultural methods are not reliable enough for routine diagnosis in all bulls. The inoculation of test heifers, with material taken from suspect bulls, is a very expensive and time-consuming procedure. The fluorescent antibody technique provides a promising method which would be much more rapid and much less expensive.

Fluorescein conjugated rabbit gamma globulin against Vibrio fetus has been prepared. It has been tested on pure cultures of V. fetus and found to stain these organisms satisfactorily. Hyperimmune gamma globulin has been prepared against other species of vibrio and common contaminants. This will be used to insure specificity.

In the study of the incidence of vibrio fetus carrier bulls, it was found that both age and tenure were found to have a highly significant effect on the rate of infection. The frequency of infection was at a peak during the age range of 6 to 11 years, the most useful life span of AB proven bulls. The incidence of carrier status in bulls that entered the stud as young sires, increased almost five-fold as they entered their 7 to 8-year tenure period. The chance of exposure and infection increases with age and tenure.

Toxicity trials were conducted using nitrofuran drugs for the treatment of infected bulls. Furazolidone cream (1%) and Furacin solution (0.2%), have been tested in bulls at two and three times the usual number of treatment applications in an attempt to determine possible toxic effects. Six bulls (2 controls, 2 given 6 treatments, and 2 given 9 treatments) were used in this trial. Weekly semen samples were collected for 3 weeks prior to the start of the treatments, and were continued on all bulls until 10 weeks after the end of the treatments. No deleterious effects on the penile mucosa, semen quality or health of the bulls were observed. Semen volume, motility, concentration, and morphology were determined on all samples collected. Semen from all bulls remained normal throughout the experiment. (New York) (ADP a1-9(Rev.)

C. Tuberculosis

Research was continued at the Michigan State University under two contracts with the USDA. Reports submitted are as follows:

(Contract No. 12-14-100-6852(45). During this year 27 calves which did not react to avian or mammalian tuberculins or Johnin were purchased. The herds of origin of these animals were reported as free of tuberculosis by the U.S. Department of Agriculture. These animals were divided into 9 lots of 3 each. Three lots were infected with a Group III mycobacterium which induced the greatest sensitivity in guinea pigs to mammalian tuberculin: 3 lots with a Group III mycobacterium which induced greatest sensitivity in guinea pigs to avian tuberculin, and 3 lots with a Group III mycobacterium which induced greatest sensitivity in guinea pigs to Edward's purified protein derivative-Battey (PPD-B). Three animals in each of 3 lots were inoculated via the intradermal route; 3 via intrauterine route, and 3 with an aerosol of the organism. Blood samples and tuberculin sensitivity data have been, and are being obtained at appropriate intervals. One animal from each lot has been or will be sacrificed at 2, 4, and 6 months post-inoculation, or earlier, if the animal's condition necessitates. Tissues from each animal were, or will be examined bacteriologically and pathologically to determine the infectivity of the organisms.

Serums collected are being examined for polysaccharide and phosphatide specific antigen. The precipitinogenic relationship of mycobacteria of human and animal origin has been investigated using antiserums produced in rabbits. Selected strains (24) of mycobacteria of human and animal origin have been examined for specific lipids by chromatologic fractionation and infra-red spectrophotometric analysis.

Studies on the change in virulence in selected atypical isolants induced by repeated passage through guinea pigs are in progress.

(Contract No. 12-14-100-5786(45). The first work undertaken on this contract was the testing of samples of feed supplements of animal origin for the presence of acid-fast organisms. To date 107 samples have been examined.

Sixty animals to be used in the study were obtained from a herd in which no tuberculin reactors were found. In addition, the animals purchased had no detectible response to avian or mammalian tuberculins or Johnin. When tested with these products in the cervical region, the animals were transported to and are maintained in the barn used for the study in such a way as to prevent their contamination by other animals.

The work has progressed according to the schedule presented in the first semi-annual progress report of this project. More specifically, the animals were first tested for the presence of internal parasites, leptospirosis, and brucellosis. Then they were tuberculin tested, using mammalian tuberculin in the caudal fold. On February 13, the feeding of the experimental ration was started. Group A (15 animals) were fed the control ration; Group B (15 animals) the control ration with killed Mycobacterium bovis added; Group C (15 animals) the control ration with killed Mycobacterium avium added, and Group D the ration containing meat and bone scrap and steamed bone meal as detailed in the previous report.

Caudal fold tuberculin tests using mammalian tuberculin were performed on different lots of 5 animals of each group at 20, 30, and 40 days following the start of feeding the experimental rations. All animals were tested with mammalian tuberculin injected into the caudal fold after being fed the experimental rations for 100 days. (Michigan) (ADP al-13(Rev.)

D. Mucosal-Respiratory Disease-Complex

Research studies were continued at the National Animal Disease Laboratory. A soluble antigen present in infectious tissue culture fluids was separated from the infective virus particle by ultracentrifugation of two serologically related strains of bovine viral diarrhea viruses, NADL-MD and Oregon C24V.

Neutralizing antibodies against the two viruses were absent in four hog cholera antisera, but present in significant titer in a commercially prepared antiserum. Precipitin tests utilizing the agar double diffusion technique formed a single line of identity between the concentrated soluble antigen of both viruses and NADL-MD and hog cholera antisera. No lines were observed using concentrated virus pellet, noninfected embryonic bovine kidney cell antigens, specific pathogen-free calf serum or swine sera.

The relationship between the antigens of bovine viral diarrhea and hog cholera were investigated cooperatively with the Hog Cholera Project. Specific staining of antigen within bovine embryo kidney tissue culture cells, infected with either Oregon C24V or NADL-MD bovine viral diarrhea virus, was accomplished using fluorescein-conjugated swine anti-hog cholera or bovine anti-viral diarrhea globulin. Also specific staining of antigen within pig kidney tissue culture cells, infected with hog cholera virus, was accomplished using the same two types of conjugates. Specificity was confirmed by appropriate controls.

It was found that immunofluorescence was a convenient and sensitive method for determining an antigenic relationship between hog cholera and bovine viral diarrhea viruses. (NADL)

Colorado State University, Fort Collins, under a cooperative agreement with the USDA, made investigations which were reported as follows: Studies were conducted on the longevity of immunity to infectious bovine rhinotracheitis (IBR), presently considered in the Mucosal-Respiratory Disease-Complex. During the past year, the serum neutralization titers of cattle which are kept in the isolation units did not show lowering of titer. There was no difference in serum titers between the group which was infected intratracheally and the group which was infected intramuscularly.

In studies on the susceptibility of mule deer to IBR, 18 of 50 deer, obtained from different areas in Colorado, were found to have significant antibody titer of IBR, 12 of them had equivocal titers and 20 of the deer were free of IBR titer. Twelve of the 20 deer were used for testing the susceptibility of IBR. These deer with significant titer of IBR were also challenged with IBR virus to see whether it would produce any clinical reactions. The results were negative. In addition to the deer, 3 elk and 1 antelope were also inoculated with IBR virus, but they did not show any clinical reactions.

Two to 4 days after injection of IBR virus intratracheally, clinical reactions were shown among the deer. Generally they showed anorexia, depression, excess salivation and respiratory distress such as increased respiration rate, dyspnea and occasionally dry cough. Two of the deer also showed excess serous nasal discharge. One deer showed conjuncto-keratitis 4 weeks after infection at which time the animal was normal otherwise. All the clinical signs were milder than those of cattle. Hematological values of white blood cells and differential counts were within the normal range.

Virus was isolated from the nasal secretions for 7 days after inoculation of virus. Virus was also isolated from the deer with conjuncto-keratitis from the eye swab. One virus isolation was obtained from the fecal swabs from another deer. This virus isolated from the fecal swabs was obtained from a deer also 4 weeks after injection of virus while there was no sign of sickness which could be observed. No specific clinical reactions were observed after challenge, which was 5 weeks after initial infection. There was no death loss resulting from IBR infection.

The pattern of serological response was similar to that of cattle. The measurable antibody titer appears 5-7 days after infection. It took 10-12 days to reach the height of the antibody level of $10^{4.5}$. The results obtained show evidence that deer are susceptible to and play a role in the epizootic of IBR.

In a study of the pathology of IBR in relation to abortion, five groups of pregnant cows, with 5 head for each group, were used for pathological and virological studies. Ten additional cattle were used as controls.

Group	No. of cattle	Stage of pregnancy	Time between infection and fetal material collected
I	10	1st-3rd trimester	---
II	5	1st trimester	3½-5 weeks*
III	5	1st trimester	4 weeks
IV	5	3rd trimester	2 weeks
V	5	3rd trimester	3-4 weeks*
VI	5	1st trimester	1 week

*Two abortions occurred after infection and 1 dead fetus found in uterus.

The abortion occurred in both first and third trimester pregnancy. The approximate time for producing abortion after infection with IBR virus in pregnant cows is approximately 3-5 weeks. Due to the spontaneous abortions in Group II and V, the rest of the pregnant cows in those groups were sacrificed, so that the specific pathological changes could be traced. During this process, one dead fetus was found from each group which gave more convincing evidence that the fetal materials were obtained close to the abortion. Materials collected are being prepared for pathological study and virus isolation. (Colorado)

Purdue University, Lafayette, Indiana, under a cooperative agreement with the USDA, reported sporadic cases of acute virus diarrhea and mucosal disease continue to occur in Indiana. Serums obtained from such herds contain high titers of neutralizing antibody against the C24v strain of virus.

Typical cases of experimental virus diarrhea followed the inoculation of susceptible calves with tissues from two of three field cases of suspected virus diarrhea. In addition to demonstrating the current presence of active virus in the herds of origin these trials provided two new isolates of virus diarrhea agent which will be tested for immunologic relationships with known strains and possible cytopathogenicity.

An agent which is cytopathogenic for ovine thyroid cell cultures was propagated in these cells during the serial passage of VD 46 virus. Subsequent study suggests that the cytopathogenic agent is not VD 46 virus or a bacterium. The identity of this (viral?) agent has not been established.

A metabolic inhibition test for the assay of polio virus was adapted to the assaying of VD-MD viruses. Virus effects were noted only when undiluted and 10^{-1} dilutions of virus were employed and so the sensitivity of the assay under the conditions employed was judged to be impracticable.

The development of a passive hemagglutination test for the detection of antibodies against VD-MD was attempted for its obvious advantage as a diagnostic test and as an aid to laboratory study of this disease complex (cross protection tests in animals and neutralization tests in tissue culture are definitive but costly and time-consuming). The test system developed employs C24v virus grown in bovine embryonic kidney cell cultures and tanned and labelled sheep red blood cells. Thus far only low titer agglutination reactions have been observed with selected field sera and with experimental sera with cells labelled with concentrated soluble antigens. However, these low titer reactions were observed only in post-inoculation samples, so studies are in progress utilizing other strains of VD-MD virus and a wider range of experimental and field case serums.

Paranatal hematologic values for Caesarean-derived calves are reported. The major developmental changes appeared to be complete by weeks 8 to 10. The lack of immature neutrophils at weeks 8, 10, and 12 indicated a relative freedom from bacterial infections. Total serum proteins increased throughout the first 12 weeks with marked changes in the relative percentages of the globulin components.

Birth weight, 180-day weaning and yearling weights with average daily gains are presented for the SPF cattle herd. This limited data indicates that the major differences in this regard, between Caesarian-derived and second generation calves, occurs prior to weaning.

A bacteriological survey directed to the detection of selected bacterial pathogens was conducted in the SPF cattle herds. Forty animals were evaluated in March, 38 in April, and 32 in June. Three specimens were taken from each animal each month - nasal swabs, eye swabs, and fecal specimens. Large numbers of several species of bacteria were isolated but during the period of this survey none of the animals appeared to be harboring bacteria known to be capable of inducing disease in cattle. This appeared to be true even though herds on surrounding farms continued to present a history of diseases associated with the specific bacteria sought in this survey. (Indiana)

Iowa State University, Ames, under a cooperative agreement with the USDA, reported work on a new serological strain of virus diarrhea virus. Identification: The new strain of virus diarrhea virus referred to as MDI-2 was a distinct serotype of virus diarrhea. The MDI-2 strain, however, does share a common soluble antigen with other virus diarrhea strains. This common antigen can be detected by the fluorescent antibody technique.

It is evident that the new strain is serologically distinct because of three reasons - 1) reciprocal cross protection tests with MDI-2 and other virus diarrhea agents failed to demonstrate cross protection: 2) specific rabbit prepared antisera to the MDI-2 agent and to C-24-V virus prototype failed to show reciprocal cross neutralization, and 3) vaccination with MDI-2 failed to protect pigs from a subsequent challenge with hog cholera virus.

Characterization: Production of Disease in Calves: MDI-2 produces a very distinct experimental disease in calves inoculated intravenously. The reaction is characterized by a diphasic temperature response with a concomitant leucopenia associated with the primary temperature elevation. Clinical signs of anorexia, dyspnea, diarrhea, increased lachrymation, and salivation were present.

Properties of the Virus:

1. Structure and classification: Electron microscopic studies revealed that the virus has a structure and a developmental cycle identical to that of myxoviruses in general. Since our antigenic studies revealed that the MDI-2 agent is related to other strains, we now conclude that the entire group of virus diarrhea agents are myxoviruses.

2. Ether sensitivity: The MDI-2 agent is ether sensitive which would support the fact that it is a myxovirus since all members of this group are ether sensitive.

3. Antigenic characteristics: We have demonstrated by a combination of serum neutralization trials, fluorescent antibody tests, and double diffusion studies in agar, that two viral antigens exist. V soluble antigen appears to be responsible for cross fluorescense studies with other agents which are not related insofar as they do not cross neutralize. The soluble antigen will react with anti C-24-V antiserum in a double diffusion test.

4. Growth characteristics: MDI-2 virus adsorbs to testicular cell monolayers rather slowly. A 3-hour adsorption time was necessary to insure 98 percent adsorption. By fluorescent antibody studies, we have determined that the first antigens appear within the nucleus at 3 hours. By electron-microscopy, these antigens may be identified as the nucleocapsid which most probably contains ribonucleic acid and protein. The fluorescence gradually disappears from the cell nucleus and appears in the cytoplasm, although there is a period where both nuclear and cytoplasmic fluorescense is seen. The intense fluorescense observed at 10 hours is correlated with densely packed complete virus particles adherent to the cell surface as observed through the electron microscope.

Virus penetration is passive in that the virus particles are apparently taken in by the normal pinocytotic process. The viral envelope is intimately attached to the plasma membrane lining the vesicle and is apparently broken

down within the vesicle. All of these events could be observed in electron-photomicrographs.

It is obvious both from fluorescent antibody studies and electron microscopy that the virus is held at the surface of the cell prior to release which is typical of myxoviruses in general.

This work has presented some very important considerations both from an applied aspect and from a very basic concept. Since this agent has been shown to be a new serological strain of virus diarrhea, it follows that the existing vaccines which only incorporate one virus would not fully protect animals against virus diarrhea.

From a basic aspect it was shown that the MDI-2 agent is related to other virus diarrhea strains by a common soluble antigen. We have also shown that this cross reaction does not exist with the noncytopathogenic virus diarrhea strains. This brings up the interesting point as to the mechanism of cell-killing by the virus. It may be that the presence of the soluble antigen actually kills the cells and that the absence of it is correlated with the noncytopathogenic strains. This idea does have a practical side because of the fact that MDI-2 strain actually is more virulent than the noncytopathic Sanders Agent.

Viral Isolation Attempts from Cattle: It was shown experimentally that the MDI-2 agent produces signs referable to both the digestive tract and the upper respiratory tract. This agent was reisolated from a nasal swab taken from an experimentally infected animal. Attempts to isolate agents from herds clinically affected with mucosal disease were negative. These included isolation attempts from 78 animals. Efforts to isolate agents from three "normal" herds were successful. The agents are being studied.

Pathology of experimental virus diarrhea: A comparative study of the pathology produced by 6 mucosal disease-viral diarrhea agents in calves under experimental conditions has been described. Four of the agents produced similar lesions in the digestive mucosa and lymph tissues which corresponded to the early lesions reported for field cases of mucosal disease-viral diarrhea. Two of the agents, which later were identified as strains of infectious bovine rhinotracheitis virus, produced similar but not as severe lesions as the other 4 agents. In addition, these agents produced multiple foci of necrosis in the adrenal cortex.

Considering the similarity of the clinical syndrome, clinical pathology, immunological protection and lesions produced, it would appear that 4 of the agents studied are closely related. The other 2 agents also appear to be related to each other but not to the other four. (Iowa) (ADP a1-14(c)(Rev.)

E. Mastitis

The work conducted at the National Animal Disease Laboratory, Ames, Iowa, was reported as follows: Twenty-five dairy cows with udders free of hemolytic, coagulase-positive staphylococcic infections were tested for blood serum alpha and beta antitoxins for periods up to 2 years. The number of serums positive for these antitoxins and the average antitoxin titers of the positive serums increased with the age of the animals and reached maximum levels during the second lactation period. However, a progressive increase in antitoxin titers during the test period was not apparent when animals were considered individually. In most animals, the titers developed to certain levels and remained relatively stable or declined to levels below 1 international unit (I.U.) of antitoxin.

Three laboratory strains of Streptococcus pyogenes were cultured by daily transfer in a medium composed of 22 amino acids, 2 purines, 1 pyrimidine, B vitamins, inorganic salts and buffered with 0.1 M phosphate buffer, pH 7.0. The basic medium is that described by Williams, Cornell Exp. Sta. Bull. No. 337 (1955). It was modified by the addition of L-glutamine, ammonium acetate and 0.1 M phosphate. The extra buffer increased growth more than 2-fold. Daily transfers of a 1 percent inoculum resulted in good growth. Optical densities measured in 24 hours with a Klett-Summerson colorimeter and 550 mμ filter were 0.40-0.50. One percent glucose was completely fermented to quantitative yields of lactic acid. (NADL)

At the University of California, Davis, under a cooperative agreement with the USDA, a strain of bacteria called Aerobacter aerogenes has been employed which may occur in the environment of dairy cattle. It was selected because it can be readily identified and at the same time will serve as a representative of the fecal (manure) bacteria. Such bacteria, called coliform organisms, are not commonly disease-producers but under certain circumstances of commercial dairying, may enter the mammary gland and produce a very severe inflammatory disease which may lead to death of the cow.

Investigations at California have shown that severe disease results only when a large bacterial population has been produced within the mammary gland. The effects on the cow are not directly related to bacterial growth but rather to destruction of the organisms by the defenses of the body. During multiplication of the organism in the mammary gland, there are no outward signs of the disease, but in time, a reaction sets in (inflammatory) which immediately destroys the organism in large numbers thereby releasing a poisonous substance in high concentration (endotoxin); it is this material released from the bacteria which produces peracute mastitis and which may even lead to death of the cow.

The production of a mild irritation of the mammary gland prior to exposure to the coliform bacteria prevented growth of the organism and the development of severe mastitis. The most important factor in a response to irritation

to mammary tissue is an infiltration of cells (leukocytes) from the blood. It was shown that cells infiltrating into the milk at levels generally accepted as representative of high normal values (250,000 to 500,000 per cc) were capable of inhibiting growth of coliform organisms within the udder and therefore were highly protective against coliform mastitis. The practical implications are that as cows become older and their mammary tissues respond to oft-repeated irritation inherent in the milking process, the leukocyte activity so engendered also at the same time protects the cows against coliform organisms. Therefore, extensive use of antibiotics to reduce mild inflammatory reactions would appear to be inadvisable. (California) (ADP a1-15(R)

F. Epizootic Bovine Abortion

The University of California, Davis, under a cooperative agreement with the USDA, reported that during the past year the following findings were made in studies of epizootic bovine abortion (EBA): 1) ingestion, as hitherto believed, does not appear to be the manner in which the virus gains entrance to the body under field conditions: 2) cattle do not become refractory to abortion following exposure, as virgin heifers, to virulent virus: 3) inactivated EBA virus vaccines do not protect cattle against abortion, and 4) the EBA virus appears to be identical with, or closely related to, the virus of enzootic abortion of ewes (EAE).

These findings suggest that the EBA virus is venereally transmitted and an infection immunity develops following exposure. However, it does not develop rapidly enough to prevent abortion in the initial pregnancy. Thereafter, cattle are refractory to reinfection and abortion with this virus because the infection immunity becomes fully operative after termination of the initial pregnancy. (California) (ADP a1-21)

G. Paratuberculosis (Johne's Disease)

Research workers at the National Animal Disease Laboratory, Ames, reported that studies were conducted to devise an improved technique for primary isolation of Mycobacterium paratuberculosis. Trypsin digestion of infected intestinal mucosa, followed by decontamination with IN NaOH, was effective in preparing the inoculum for primary cultivation. A lymph node-egg yolk medium was superior to several other mediums for primary cultivation and subcultivation of newly isolated strains.

A herd of cattle, ranging in size from 161 to 195 head, in which Johne's disease was known to exist, was tested periodically with intradermic johnin. Selected tissues of all animals removed from the herd were examined after slaughter for Mycobacterium paratuberculosis. The following observations were made on 96 animals eliminated from the herd during this 5-year study:

Forty-six cattle reacted to intradermic johnin. Fifteen of the reactors developed clinical evidence of Johne's disease, and 21, including these 15, were found to be harboring M. paratuberculosis after slaughter.

Twenty of 50 nonreactors were also found to be harboring the bacillus after slaughter, and 10 of these had developed clinical evidence of Johne's disease; a total of 20 cattle, including these 10, were found to be harboring M. paratuberculosis.

Twenty-six cattle that reacted to intradermic johnin were tested periodically for several years, and the following observations were made: a) sensitivity persisted for only 6 months in 12 cattle, 3 of which were found to be infected with M. paratuberculosis when examined after slaughter: b) Sensitivity persisted for 12 months in 5 others, 1 of which was found to be infected when examined after slaughter: c) sensitivity persisted for 36 months in 5 cattle, 3 of which developed clinical evidence of Johne's disease: d) sensitivity was intermittent with no particular pattern in 4 cattle. One of these animals developed clinical evidence of Johne's disease, but in the others there was no evidence of the disease when examined after slaughter. (NADL) (ADP al-35)

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AREA NO. 2 - INFECTIOUS AND NON-INFECTIOUS DISEASES OF SWINE

Problem. Profitable swine production depends largely on the ability to control diseases. Swine diseases cause losses estimated at more than $200 million annually. In order to control and eventually eradicate these diseases, a thorough knowledge of causes, diagnostic procedures, preventive procedures, and treatments is required. Although a great deal of excellent research has been and is being accomplished, a vast amount of research is still required to obtain this knowledge. At present, the causes of several important swine diseases are unknown or incompletely understood. Extensive fundamental research on swine diseases is essential to the welfare of the swine industry.

USDA PROGRAM

The Department has a long history of swine disease research. For example, research on hog cholera was initiated in 1884. Research on this and other important swine diseases is a continuing long-term program. Modern research techniques in the areas of biochemistry, biophysics, pathology, microbiology, pharmacology, physiology, and immunology, are being applied to swine disease problems. Research is being conducted on the following diseases at the designated locations.

The Federal scientific effort devoted to research in this area totals 23.3 professional man years. This effort is divided among sub-headings as follows:

Hog Cholera 9.1 at the National Animal Disease Laboratory, Ames, Iowa, the Florida Hog Cholera Research Station, Live Oak, Florida, under a cooperative agreement with the University of Illinois, and under a contract with the University of Nebraska.

Atrophic Rhinitis 4.0 at the National Animal Disease Laboratory, Ames, Iowa.

Transmissible Gastroenteritis 3.6 at the National Animal Disease Laboratory, Ames, Iowa, and under cooperative agreements with Purdue University and the University of California, and a memorandum of understanding with the University of Illinois.

Erysipelas 3.6 at the National Animal Disease Laboratory, Ames, Iowa, under a cooperative agreement with the Department of Biochemistry, Seton Hall College of Medicine and Dentistry, Jersey City, New Jersey, and in connection with a PL 480 grant to the Institute for Veterinary Research, Pulawy, Poland.

Brucellosis 3.0 at the National Animal Disease Laboratory, Ames, Iowa.

REPORT OF PROGRESS FOR USDA AND COOPERATIVE PROGRAMS

A. Hog Cholera

Hog cholera research at the National Animal Disease Laboratory, Ames, Iowa, was conducted in the following phases:

Propagation of hog cholera virus in vitro. A method of identifying hog cholera virus in animal tissues by the use of immunofluorescence has been developed. The virus is grown in swine kidney cells and stained with a fluorescence stain and examined under a special microscope to demonstrate the virus-infected cells. This test has proved about 98 percent efficient in detecting hog cholera in experimental animals. The efficiency of this test in diagnosing field cases of hog cholera is being investigated.

Antigenic relationship between hog cholera and bovine viral diarrhea. Specific staining of antigen within bovine embryo kidney tissue culture cells, infected with either Oregon C24V or NADL-MD bovine viral diarrhea virus, was accomplished using fluorescein-conjugated swine anti-hog cholera or bovine anti-viral diarrhea globulin. Also specific staining of antigen within pig kidney tissue culture cells, infected with hog cholera virus, was accomplished using the same two types of conjugates. Specificity was confirmed by appropriate controls.

Agar-gel diffusion studies for diagnosing hog cholera. A comprehensive investigation of this serological procedure gave no evidence that the observed precipitation reactions were related to hog cholera virus and its corresponding antibody. (ADP a2-17(C))

Pilot hog cholera eradication field studies to evaluate hog cholera vaccines. The evaluation of experimental field trial hog cholera eradication, using modified live virus vaccines, has continued in Suwannee County, Florida, from April 1957 to December 31, 1962. During this period the study was designed to measure the potency, safety, and shelf life of three types of commercial modified live-virus vaccines (lapine, porcine, and tissue culture origin) administered with a minimum of 15 ml of anti-hog cholera serum. Records were made of all vaccinated and unvaccinated swine herds, purchased or raised, and approximately 4.5 percent of the vaccinated swine were challenged with virulent virus at market age to measure their immunity from vaccination. All suspected hog cholera outbreaks in the county were investigated and swine inoculation tests made for confirmation of the disease virus.

Since December 31, 1962, the field trial study has been changed to measure the spreading characteristics of modified live virus vaccines administered with and without hog cholera antiserum.

During the period 1957-1962, data collected from challenge of 4073 swine vaccinated with 87 serial numbers of lapine origin, porcine origin or tissue

culture modified live virus vaccines from 11 licensees show an inverse relationship between average age of vaccine at time of use and immunogenicity of vaccine. Hog cholera was confirmed in six vaccinated, farm-raised swine, in nine non-vaccinated, farm-raised swine, and in two vaccinated, purchased hogs. The difficulty of enforcing regulations restricting movement of non-vaccinated swine from public market premises has been described. Adherence to quarantine provisions by most swine-raisers and effective enforcement of regulations affecting swine transportation by the Florida Division of Animal Industry have been reported.

During fiscal year 1962, a similar field trial cooperative study with the Animal Disease Eradication Division of ARS and the State of Georgia, was started in Lowndes County, Georgia. This pilot plant study is being made to evaluate inactivated or killed hog cholera virus vaccines as an eradication tool. The study has been in progress for over 18 months without the development of a single confirmed case of hog cholera. (ADP a2-13)

The Hemagglutination test for diagnosing hog cholera. This investigation is being carried out under a cooperative agreement at the University of Illinois. Evaluation of the hemagglutination test has shown that it is not sufficiently reliable, in its present form, to be used for routine diagnosis of hog cholera. It appears that the major difficulty is the tendency of the formalinized erythrocytes to agglutinate spontaneously. Work is continuing to improve specificity through the preparation of a more stable erythrocyte suspension and by purification of hog cholera virus and concentration of hog cholera antibodies. (ADP a2-17(C))

B. Atrophic Rhinitis

In studies of atrophic rhinitis at the National Animal Disease Laboratory, 113 swine were examined with a rhinoscope. The rhinoscopic examination was compared with a subsequent postmortem examination. Both examinations had a 60.1% agreement for the degree of atrophy, which included 86.0% agreement of the negatives but only 16.7% of the positives. According to biometric analysis, the correlation between the postmortem and rhinoscopic scores for this group was +0.75.

The results indicate that the rhinoscope may have some value as a diagnostic tool but is not reliable for research purposes. (ADP a2-8(Rev.)

C. Transmissible gastroenteritis (TGE)

Research investigations on TGE at the National Animal Disease Laboratory were continued with emphasis on the development of methods for diagnosis, prevention and control. This work has not, as yet, progressed to the reporting stage.

At the University of California the interrelationship of swine enteroviruses was investigated. (ADP a2-10(Rev.)

At Purdue University investigations were carried out on attempts to propagate TGE virus in laboratory animals, embryonating chicken eggs, and tissue and organ cultures. In addition, the mechanism whereby immunity is transferred from sows to pigs was investigated. (ADP a2-10(Rev.)

D. Swine erysipelas

A type-specific antigen has been isolated from acetone dried cells of Erysipelothrix and after extensive purification has been shown to be composed of a polymer of hexosamines to which peptide components are attached. This mucopeptide antigen is most probably derived from the cell wall of the organism. The antigen isolated in this manner is identical both chemically and serologically with the antigen present in acid extracts of the organism. It was acid extracts of cells of Erysipelothrix which were used originally to establish types and strains of the organism. (ADP a2-15)

E. Brucellosis

Work on swine brucellosis at the National Animal Disease Laboratory was carried out on the serology, bacteriology and histopathology of this disease. This work has not, as yet, progressed to the reporting stage. (ADP a2-16)

F. PL 480 Project

1. Studies on the Antigenic Structure of Erysipelothrix rhusiopathiae.

This investigation is carried out under a PL 480 Grant to the Institute for Veterinary Research, Pulawy, Poland. The work, still in the preliminary stage, is aimed at improving diagnostic and immunizing procedures.(E21-ADP-8)

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AREA NO. 3 - INFECTIOUS AND NON-INFECTIOUS DISEASES OF SHEEP AND GOATS

Problem. There are at least 18 infectious diseases of sheep and goats in the United States that cause an estimated annual loss of 15 million dollars. Non-infectious diseases are estimated to cause an additional 3 million dollar loss annually. The cause of some of these diseases is known; others have more than one causative agent contributing to produce the effects seen in field cases. Environmental, genetic, and unknown factors appear to play a part in some diseases. The natural reservoirs of the known infectious agents have not been fully determined. Fundamental information on methods of transmission and means of prevention are needed for many of these diseases. Vaccines and other immunizing products are available for some diseases of sheep but not for others. Some of these products might be improved. Prevention, control, or eradication of disease is necessary for economic and efficient sheep and goat raising. Due to lack of accurate, rapid diagnostic techniques, infectious diseases often get a substantial start in a band or flock before they are recognized, partly because they are easily confused with non-infectious diseases.

USDA PROGRAM

The Department has a continuous long-term program involving biochemists, microbiologists, pathologists, and veterinarians engaged in both basic studies and the application of known principles to the solution of infectious and non-infectious diseases of sheep and goats. Research is being conducted on the diseases at the following designated locations.

The Federal scientific effort devoted to research in this area totals 7.6 professional man-years. This effort is applied as follows:

Bluetongue, 2.0 at the Denver Animal Research Laboratory, Denver, Colorado.

Contagious Ecthyma, 2.0 at the National Animal Disease Laboratory, Ames, Iowa.

Foot Rot, 2.0 at the National Animal Disease Laboratory, Ames, Iowa.

Vibriosis, 0.3 under cooperative agreements with the Colorado, Montana, and Utah Agricultural Experiment Stations.

Scrapie, 0.2 at the Agricultural Research Council Field Station, Compton, Berkshire, England, and the Moredun Institute, Edinburgh, Scotland, through two grants of PL 480 funds, equivalent to $300,165. The work is coordinated through the European Mission for Research on Animal Diseases, Amsterdam, Holland.

Viral Ulcerative Dermatosis, 0.1 through a cooperative agreement with the Colorado Agricultural Experiment Station.

Paratuberculosis or Johne's Disease, 1.0 at the National Animal Disease Laboratory, Ames, Iowa.

REPORT OF PROGRESS FOR USDA AND COOPERATIVE PROGRAMS

A. Bluetongue.

During the reporting period, July 1, 1962 to June 30, 1963, at the ADP Denver Laboratory, sheep blood samples were tested representing suspected bluetongue (BT) outbreaks in 24 bands and flocks from 8 States, all of which were found to be infected. Cattle blood samples of 15 herds from 5 States were tested for BT virus and found positive.

Thirty one lambs and 12 adult ewes were infected with BT virus either by intravenous, subcutaneous, or intradermal inoculation. The intradermal route was the route of choice for infecting sheep since it is analogous to the route by insect bites and only relatively small dosages were required to elicit a typical BT clinical response. The characteristic clinical responses of the BT infected sheep in their usual chronological sequence were panting; hyperemia of the skin of the muzzle, lips, and ears; elevated body temperature; inflammation, swelling, and ulceration of the oral mucous membranes; anorexia; depression; aspiration pneumonia; coronitis and death. The onset of these signs and symptoms occurred on day after inoculation (DAI) 1-7 and commonly terminated by DAI 14 in the lambs and by DAI 21 in the adult sheep. Emphasis was placed on the correlation of hemogram changes with the clinical manifestations and necropsy observations. A leukopenia developed in all of the infected sheep. In young sheep the average maximum leukopenia occurred 48 hours prior to the average peak body temperature, while in adult sheep they both occurred on DAI 7. Also, lambs had their average peak body temperature on DAI 7. A neutropenia, eosinopenia, and lymphopenia occurred in 86% and a hemolytic anemia was observed in all but one of the experimental sheep. The neutropenia and eosinopenia developed during the convalescing stages of the infection, while the lymphenia paralleled the leukopenia.

Work was continued at the Denver Laboratory, in cooperation with the Entomology Research Division, in studies on the role of insects as transmitters of virus diseases. The results obtained from a series of 5 experiments indicate that the sheep ked, Melophagus ovinus (L.) can transmit the virus of bluetongue disease in sheep. Ten of the 18 principal BT susceptible sheep used in the experiments on which sheep keds were manually transferred from a BT host virus sheep, were positive, 6 questionable, and 2 negative for clinical evidence of BT disease. Five of the above 10 positive sheep had mild infections. Fifty percent of the questionable reacting sheep and 60% of the mildly reacting sheep had highly intensified clinical BT reactions when challenged with the standard BT virus. Five of the 10 positive reacting sheep were immune following their challenge with known BT virus. Blood

obtained from 2 of the positive reactors was subpassaged into BT susceptible sheep and they reacted with typical signs and symptoms of the disease.

A procedure has been developed at the Denver Research Laboratory for counting sheep leukocytes and erythrocytes by electronic means. A device and technique has also been developed for handling quantities of coverslips used in fluorescent antibody and other cell culture work. A technique and apparatus were designed for collecting large quantities of sterile blood quickly without hemolysis. (Denver, Colorado)

Using the bluetongue virus as a tool for virus study at the Fur Animal Disease Laboratory, Pullman, Washington, it was found that the relationship of mouse age to virus susceptibility was influenced by the passage level of virus used. Virus of the 7th passage produced mortality in one, but not in two-week-old mice, whereas virus representing the 53rd passage killed 5-month old mice. Peak titers were recorded at approximately the same time in the different age groups, but were inversely related to mouse age. A marked prolongation of survival times was observed in 3- to 20-week-old mice as compared to one-week-old mice.

Analysis of graded response data showed the distribution of survival times for suckling mice to be approximately normal, but for mice one and one-half to 3 months of age the same distribution was positively skew with 20 per cent of the animals demonstrating increased resistance. The two distributions had equal standard deviations.

A study of virus multiplication in one and one-half to 3-month-old mice revealed an earlier reduction of infectivity in brains of surviving mice than in mice that succumbed to the infection. Late spread of virus to important neural sites or increased sensitivity to the virus are possible explanations for deaths following markedly prolonged survival times. The pattern of virus multiplication in older mice disagrees with the concept of the incubation period as a time of steady virus multiplication in infected organs.

The interfering effect of high doses of active egg-propagated bluetongue virus upon mouse-adapted virus resulted in no reduction in mortality but markedly prolonged survival times. The failure of absolute interference most likely was due to a dual infection with immunologically related viruses in the same cells. The results indicate that the site of interference is one of the late steps (virus synthesis or release) in the multiplication cycle. (Pullman, Washington) (ADP a3-5)

B. Vibriosis in Sheep.

In work under a cooperative agreement with the Colorado State University at Fort Collins, two year-old primigravid ewes were used to determine the efficacy of killed Vibrio fetus serotypes I and V organisms to protect sheep against measured dosage of (a) serotype I culture challenge, (b) serotype V culture challenge, and (c) serotype I and serotype V (1:1 ratio) culture

challenge. Immunity was challenged during advanced gestation. One abortion occurred in 21 ewes vaccinated with serotype I and serotype V organisms when challenged with serotype I organisms. No abortions occurred in 40 ewes similarly vaccinated when challenged with serotype V organisms, or the combined serotype I and V challenge inocula. Fifteen abortions (65.2%) occurred in 23 nonvaccinated, serotype I challenged control ewes, while four abortions (19.0%) occurred in 21 nonvaccinated serotype V challenged control ewes. (Fort Collins, Colorado)

In cooperation with the Montana Veterinary Research Laboratory of the Montana Agricultural Experiment Station at Bozeman, work has continued on serotype change. A total of 91 ewes were on an experiment conducted in the Spring of 1963 to obtain data on the possibility of serotype changes. Forty-two cultures of V. fetus were isolated from aborted fetuses and used to make antigens. Serotyping has not been completed. Studies were made on the significance of intestinal infection with Vibrio fetus.

Cultures of V. fetus intestinalis were obtained from Belgium and studied. Initial attempts to isolate V. fetus from previously inoculated ewes were not successful. Better results were obtained later using brilliant green-blood agar. A total of 12 isolations of vibrios, 10 of which were V. fetus, were made from 4 ewes during a period of 11 days following rumen injection.

The significance of placental infection with V. fetus was studied in normally lambing ewes. A total of 176 placentas from a ranch with a previous history of vibriosis (1960, 1962) were obtained for culture and two isolations of V. fetus were made. Insofar as could be determined, both isolations were made from the placentas of normal lambs. It would appear that vibriosis has been maintained on the ranch since 1960, although the flock appeared to lamb normally in 1961 and 1963.

Additional work was done on the identification and propagation of V. fetus. Temperature tolerance at 25°C, 37°C, and 42°C was determined for a number of V. fetus cultures of various origins. This procedure has some promise for identification. A simple method for the production of heavy growth of bovine and ovine V. fetus cells was developed. (Bozeman, Montana)

In cooperation with the Utah State University, Utah Agricultural Experiment Station, at Logan, the work was continued on studies on vaccination. The replacement ewes of two bands with a total of about 2,000 ewes each, were vaccinated for the third year with a vibrio vaccine. One band had an abortion rate of 2.1 per cent and the other 3.5 per cent. No vibrio organisms were isolated from these abortions. Vaccination of the replacement ewes appears effective in eliminating Vibrio fetus infection of a given band or flock.

The effect of vibrio organisms classified as Vibrio fetus and Vibrio bubulus differing distinctly in their cellular morphology, catalese activity and hydrogen sulfide production, was studied during the first 24 hours after

intravenous inoculation of pregnant and nonpregnant ewes. All strains studied caused a rapid drop of the total leukocyte number. The lowest point was reached 3-5 hours after inoculation. The number of leukocytes increased then again and was slightly above the pre-inoculation values at the 24th hour. The vibrio organisms inoculated were removed from the blood within 30-60 minutes except when coccoid Vibrio fetus organisms were used which remained in blood up to 6 hours. Two of the ewes inoculated with the coccoid organisms died 6 and 9 hours thereafter. The vibrio organisms were eliminated from most of the organs of the ewes after 24 hours. Vibrio organisms were isolated at that time in low numbers and irregularly from brain, meninges, pancreas, skeletal muscle and duodenum. They were detected rather regularly from the gall bladder tissue and were there in numbers as high as 10^6 per Gr. of tissue. All Vibrio fetus strains were found in the uterus and uterine content at the time of necropsy 24 hours after inoculation. (Logan, Utah) (ADP a3-1(R)

C. Scrapie

Investigations of scrapie in sheep and goats has continued under the terms of the agreement at the Agricultural Research Council Field Station, Compton, Berkshire, England. An encephalopathy has been produced in mice by intracerebral inoculation of material taken from goats infected with scrapie. Pathology in the mice is similar to that in goats and sheep. The incubation period in mice is 7 months. Research with mice is continuing and it is possible that in time mice may replace goats as experimental animals in scrapie research. However, a considerable amount of additional work is necessary before a decision of this nature may be reached.

In attempts to demonstrate specific antibodies to the scrapie agent the workers have tried to hyperimmunize infected goats and rabbits with suspensions of infected brain material. All attempts to demonstrate complement fixation and virus neutralizing antibodies have failed. Attempts have also been made to transmit the disease to goats by oral doses of scrapie saliva or feces from animals infected with scrapie. From these studies it would appear that it is difficult to transmit experimental scrapie by contact among housed animals and that the agent seems unlikely to be present in saliva or feces.

It has also been found that the agent of scrapie is extremely resistant to formalin. Ten per cent suspensions of scrapie goat brain material were adjusted to contain .01, .05, 1%, 2%, 4% or 8% formalin in normal saline. After thorough shaking these suspensions were incubated for 18 hours at 37°. All inoculated animals developed the disease. There was no significant variation in incubation time between groups of goats that were infected which would indicate that formalin treatment, even in 8% concentration, had not affected the scrapie agent adversely.

More recently it has been shown that the 5th tissue culture passage material may contain a scrapie agent. One mouse inoculated with the tissue culture material died approximately 8½ months after inoculation. Thus far there has

been no evidence of the spread of the disease by contact between mice inoculated with the scrapie agent and those not so inoculated over a 168 day period of observation. Work is continuing along the lines mentioned above.

At the Moredun Institute, Edinburgh, Scotland, work has also continued under the terms of the agreement. Particular emphasis has been placed on the genetic constitution that probably determines susceptibility. The main feature is the continuing high incidence of scrapie in certain groups or blocks. These groups include those where dams and sires are from scrapie parentage (SS) or dams are from scrapie parentage and sires are from scrapie-free parentage (SF). These are compared to groups where dams and sires are from scrapie-free parentage (FF) or dams are from scrapie-free parentage and sires are from scrapie parentage (FS). (ADP a3-3)

D. Viral Ulcerative Dermatosis.

In cooperation with the Colorado Experiment Station, in vivo immunologic studies indicated that sheep immune to ulcerative dermatosis (UD) remained susceptible to contagious ecthyma (CE) and vice versa. Convalescent serums produced by either agent did not provide measurable protection when incubated with the homologous viral agent and inoculated onto susceptible sheep. Unequivocal antibody formation could not be detected in the immune serums against either UD or CE by the in vitro technique employed. The failure to positively demonstrate antibodies in the convalescent serum of sheep in this investigation precluded any possibility of studying the immunologic relationship of these two viral agents by the serologic tests employed.

The viruses of ulcerative dermatosis (UD) and contagious ecthyma (CE) were adapted to growth in monolayer cultures of bovine embryonic kidney cells. After ten culture passages, there was no demonstrable reduction in the pathogenicity of the viruses for sheep. Mice, rabbits, guinea pigs, and hamsters were refractory to scarification and inoculation with the agents of UD and CE. Both virus entities were resistant to ether.

The question of the similarity of the agents of UD and CE is not clearly defined, and there may be more agents of the ovine dermatitides complex that need investigation and comparison. The multiplicity of strains has not been adequately investigated and undoubtedly plays a major role in the various dermal infections of sheep. (Fort Collins, Colorado) (ADP a3-4)

PUBLICATIONS REPORTING RESULTS OF USDA AND COOPERATIVE RESEARCH

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AREA NO. 4 - DISEASES AND PARASITES OF HORSES

Problem. Currently there are about 3,250,000 horses in the United States, valued at approximately $860 million. About one million of these are draft animals. Considerable numbers of horses and mules are still required for work on cattle ranches and as pack animals. The annual overall value of the horse industry has been estimated at about $1.5 billion. The horse may be an important link in epizootiology of animal diseases in general. Equine piroplasmosis is an acute, subacute, or chronic tick-borne disease of horses that was first recognized in this country in Florida in 1961. It is characterized by high fever, progressive anemia, jaundice, edema, extreme weakness and depression. Fatalities range from 5 to 50 percent of infected animals. This disease, now apparently well established in Florida, has extended into Georgia and poses a serious threat to the entire equine population in the southern United States. The disease is clinically indistinguishable from equine infectious anemia. Horses which have clinically recovered from piroplasmosis usually remain carriers of the disease and are a potential source of infection. African horsesickness, a highly fatal disease of equines, that was confined to Africa until recently, is presently causing serious losses in the Middle East and parts of Asia.

USDA PROGRAM

The Department has recently started a continuous long-term program involving biochemists, pathologists, protozoologists, and veterinarians to work on equine piroplasmosis. In order to be prepared in the event of introduction of African horsesickness into the United States, the Plum Island Animal Disease Laboratory has obtained African horsesickness viruses and antiserums from South Africa. These materials are thus directly available for diagnostic and vaccine studies should the need arise.

The Federal scientific effort devoted to research in this area is 5.5 professional man-years. This effort is divided among sub-headings as follows:

Serological diagnosis, transmission, and control of equine piroplasmosis 3.2 at the Beltsville Parasitological Laboratory, Beltsville, Maryland.(In cooperation with the Entomology Research Division)

Chemotherapeutic methods of prevention, treatment, and eradication of piroplasmosis in horses 1.1 under contract with the University of Florida, Gainesville.

Development of antigenic material for a diagnostic test for equine piroplasmosis 1.2 under contract with the University of Kentucky, Lexington.

PL 480 funds have been made available in Turkey for research on Gastrophilus pseudo-hemorrhoidalis (equine parasite) in Turkey; its distribution, life cycle, economic importance, treatment and control.

REPORT OF PROGRESS FOR USDA AND COOPERATIVE PROGRAMS

The viruses and antisera for African horsesickness are being held available at the Plum Island Animal Disease Laboratory.

The work on equine piroplasmosis was not activated until May 1963, so there has not been time for any reports on progress of the various phases of investigations.

PUBLICATIONS: None

AREA NO. 5 - INFECTIOUS AND NON-INFECTIOUS DISEASES OF POULTRY

Problem. Annual losses from infectious and non-infectious diseases of poultry, exclusive of parasitisms, are estimated to be at least $200 million. Continued and expanded basic and applied research are essential to aid in reducing these losses, which inevitably affect cost to the consumer. Added to the initial losses from mortality, reduced weight gains, poor feed utilization, decreased egg production, and lowered quality, are the final losses occasioned by condemnations at dressing plants. Since institution of compulsory inspection for interstate movement of poultry and poultry products, overall condemnations because of disease have skyrocketed. The problem is to keep abreast of changing conditions in the field, which present increasingly complex problems requiring basic information.

USDA PROGRAM

The Department has a long-term program involving biochemists, microbiologists, pathologists, and veterinarians engaged in both basic studies and the application of known principles to the solution of infectious and non-infectious diseases of poultry. Research is being conducted on the diseases at the following locations.

The Federal scientific effort devoted to research in this area totals 31.4 professional man-years. This effort is applied as follows:

Ornithosis 5.1 at the National Animal Disease Laboratory, Ames, Iowa, and under cooperative agreements with the Universities of California and Minnesota, and the Agricultural Experiment Stations of Oregon and Texas.

Salmonellosis 3.0 at the National Animal Disease Laboratory, Ames, Iowa.

Pasteurellosis 2.0 at the National Animal Disease Laboratory, Ames, Iowa.

Chronic Respiratory Disease Complex 16.7 at the National Animal Disease Laboratory, Ames, Iowa, the Southeast Poultry Research Laboratory, Athens, Georgia, and under cooperative agreements with the Agricultural Experiment Stations of Connecticut, Delaware, Georgia, Massachusetts, New York, North Carolina, Texas, Virginia, and Wisconsin, and with the University of Minnesota. A basic project on chronic respiratory disease is in progress at the Hebrew University, Jerusalem, Israel, under a PL 480 Grant of funds equivalent to $29,189 over a 3-year period.

Newcastle Disease 4.2 at the National Animal Disease Laboratory, Ames, Iowa, the Southeast Poultry Research Laboratory, Athens, Georgia, and under cooperative agreements with the University of Maine and the Wisconsin Agricultural Experiment Station.

Bluecomb 0.1 under contract with the University of Minnesota, St. Paul.

Leukosis 0.3 under cooperative agreement with the Regional Poultry Research Laboratory, USDA, East Lansing, Michigan.

REPORT OF PROGRESS FOR USDA AND COOPERATIVE PROGRAMS

A. Ornithosis

Cooperative studies at the University of California showed that chickens, inoculated with ornithosis virus by different routes, failed to show signs of the disease during 4 weeks, but 65 percent of them produced complement-fixing antibodies at a low level. The virus was isolated from the spleen and liver from only one bird 4 weeks after inoculation. It was observed that not all ornithosis yolk sac antigens showed the same antigenic characteristics to fix complement with avian serums in the Brumfield's DCF test. One antigen (YS-10) that inhibited the reaction, was not affected by heat-treatment such as 56° or 100°C for 30 minutes.

A modified ICF test was conducted using rabbit immune serums as an indicator positive serum in place of human or pigeon serums. Relatively high correlation was obtained between the modified ICF and the Brumfield's DCF tests using chicken and turkey serums.

An antibody titer in the Brumfield's DCF test was affected by individual normal chicken serums normally added to the system, but 3 times concentrated normal chicken serum was not always required by chicken antigen-antibody complexes in the Brumfield's DCF test. (California)

Cooperative work at Minnesota indicated through serologic evidence, that the ornithosis entity remains prevalent in the avian population, particularly in turkeys. A field outbreak occurred on one farm involving two breeder flocks. Clinical, fluorescent antibody, serological and pathological findings were confirmed by virus isolation in embryonated chicken eggs and mice. A younger flock, six weeks of age, on the same farm never developed serological or clinical evidence of the disease.

A very good correlation between parallel tests with a bacterial extract antigen and the ornithosis antigen on large numbers of experimental and field cases has been obtained. Antibody titers could apparently be detected earlier with the bacterial antigen. The substitution of this antigen for the ornithosis virus in the DCF test could lessen the human hazard of infection and make available a simple test for use in more laboratories.

Fluorescent antibody techniques and hypersensitivity reactions indicate that both show promise as aids in the diagnosis of ornithosis.

Free flying birds tested for ornithosis antibodies have given negative results.

A cooperative study with the School of Public Health to evaluate the prevalence of DCF titers of people working on premises with turkeys has been conducted. Five and one half per cent of the individuals have suspicious titers with less than 0.5% with positive titers. On these farms, 10.6% of the turkey flocks were considered positive by the DCF test while 4.6% of the flocks showed suspicious titers. (Minnesota)

In cooperative work at the Oregon Agricultural Experiment Station, an ornithosis isolate of sea gull origin (G1) found to be highly pathogenic to turkeys, white mice and chicken embryos, was propagated by serial passage in two cultural systems, white mice and chicken embryos. The pathogenicity has increased for mice to the degree that it is killing them in 3 days, whereas earlier passages would kill in 5-6 days. The pathogenicity for chicken embryos has been quite erratic and there is no indication of increased pathogenicity at the 18th passage.

Year old Beltsville white turkey hens were challenged with the 10th and 20th serial mouse passage and the 10th serial chicken embryo passage. Each hen received 1.0 ml. of a 10% infected tissue suspension inoculated intramuscularly. The results showed that the 10th and 20th mouse passages were very pathogenic for turkeys at the dosages given. The 10th embryo passage was less pathogenic for turkeys.

Beltsville white turkeys have been immunized by exposure to a live culture of ornithosis virus of low toxicity (T3). A mild clinical response resulted from this exposure but no viremia could be demonstrated. Serologically there was a transitory reaction to Mycoplasma gallisepticum antigen 13 days postinoculation. After 27 days the birds were negative to this antigen. Ornithosis challenge with the highly pathogenic G1 isolate at 4, 7, 10, and 18 months postimmunization did not produce any clincal response or demonstrable viremia. The T3 isolate has been serially passed 16 times in mice with no evidence of any increase in pathogenicity. The isolate will infect mice but deaths occur only sporadically. Attempts to serially pass this isolate in chicken embryos have not been successful. (Oregon)

At the Texas Agricultural Experiment Station, in research work during the year, turkey ornithosis antiserum of high titer collected at various intervals after infection, was fractionated by three methods. Methods of fractionation were column chromatography, sucrose gradient ultra centrifugation and starch electrophoresis. All fractions were run by the three recognized serological tests for complement fixing antibody (indirect complement-fixation (ICF), Benedict direct complement-fixation (BDCF), and Brumfield direct complement-fixation (Br DCF).

The starch electrophoresis and sucrose gradient fractionization show that the ICF test is measuring principally a fast moving heavy antibody, whereas the DCF tests are measuring a slow moving light antibody.

Swine of various ages were refractory to ornithosis virus. This species is the first one that has not been susceptible to this agent. It is interesting to note that no lymphogramuloma venerum group agents have been isolated from swine.

Sheep (ewes) were susceptible to ornithosis when inoculated, but transmission did not occur from infected turkeys to normal sheep. (Texas) (ADP a5-20)

B. Chronic Respiratory Disease Complex

At the National Animal Disease Laboratory, Ames, Iowa, the following studies have been conducted: a) comparison of the effects of turkey Mycoplasma gallisepticum infection produced by intranasal inoculation, intratracheal inoculation and contact with inoculated turkeys; b) serological studies of Mycoplasma (PPLO) of avian origin.

Under a) above, research was conducted with Mycoplasma gallisepticum to compare effects of intranasal, intratracheal and pen contact exposure in turkeys maintained free of environmental stress. Clinical signs of infection, as indicated by coughing or tracheal rales, appeared 3 weeks after exposure and reached a peak during the 6th week when signs were observed in 70% of the birds. Antibodies for M. gallisepticum, as determined by weekly tube agglutination tests, were first demonstrated 4 weeks after exposure, and were present in all turkeys by the 7th week.

Periodic postmortem and bacteriological examination of turkeys during the course of the infection revealed that M. gallisepticum could be isolated without difficulty from trachea, lungs and air sacs until 10 weeks after exposure. None were isolated after 10 weeks. With the exception of one turkey examined early in the course of the infection, all turkeys had air sac infections. Only one turkey out of the group of 28 developed a sinusitis, and only two developed secondary bacterial infections (Escherichia coli). No differences in clinical signs, lesions, or serological response were produced by using three different methods of exposure.

Under b) above, nineteen strains of PPLO of avian origin were classified serologically into 7 groups on the basis of the tube agglutination and growth inhibition tests. The three strains, S-6, F, and PG-31, associated with chronic respiratory disease of chickens, comprised a single serological group and could be distinguished from the other strains by the serological tests.

The serological studies were done in two series of experiments. In the first studies, antisera were produced in rabbits by inoculating 6 to 10 times with PPLO antigens. The 19 PPLO strains were then grouped serologically using the tube agglutination test. In the second part of the studies, growth inhibiting antisera (requiring from 10 to 40 inoculations) were produced in rabbits. The 19 PPLO strains were grouped again serologically

using both the tube agglutination and the growth inhibition tests. Discrepancies between the two sets of antisera were observed. The more hyperimmune sera of the second studies were considered more reliable for grouping the PPLO serologically. The tube agglutination and the growth inhibition tests appeared to be of equal value where the more hyperimmune antisera were used, although neither test alone was completely reliable.

Growth inhibition and agglutination appeared to be separate activities in the antiserum complex. (ADP a5-21)

At the Southeast Poultry Research Laboratory, Athens, Georgia, the following work has been done:

Fluorescent Antibody Studies with Infectious Bronchitis Virus.

Promising results have been obtained with the fluorescent antibody (FA) technique applied to infectious bronchitis virus (IBV). Infected tissue cultures and tracheal sections fluoresce brightly with laboratory and field strains of virus. Cross reaction with Newcastle disease virus has not been observed. The FA technique was demonstrated to be sensitive enough to follow the growth cycle of IBV in tissue culture. This application will be useful in studies to adapt field strains of virus to growth in tissue culture.

Cultivation of Infectious Bronchitis Virus in Tissue Culture.

While the egg-adapted Beaudette strain of infectious bronchitis virus is readily grown on chick kidney cell cultures, attempts to adapt field strains of this virus to tissue culture have not succeeded beyond the 5th passage. However, by use of fluorescent antibody techniques some evidence of infection has been demonstrated with selected field strains.

Improved Culture Media for Growth of Mycoplasma Gallisepticum (S6 Type).

An improved culture medium for growth of Mycoplasma gallisepticum was developed. Using brain heart infusion as a base, the optimum levels of various growth additives were determined and incorporated in to the medium. Experiments on buffers showed maximum growth was obtained using Tris buffer rather than phosphate buffer. The effect of various inoculum concentrations on the volume of the resultant cell crop was subsequently determined.

Mycoplasma Gallisepticum Antigen Production.

Sufficient plate antigen was produced during the year to conduct 336,000 tests. In addition to use in our own research program, the Georgia Poultry Diagnostic Laboratory, Gainesville, has been supplied with over 250,000 test doses.

Chronic Respiratory Disease Control Program.

Condemnations due to airsacculitis were 13 times greater in progeny from a Mycoplasma-positive flock than from a Mycoplasma-free flock. Similarly, the Mycoplasma-free progeny had the best liveability, lowest mortality, highest average weight and lowest cost per pound. Feed conversions in the two groupings were identical. Over 100,000 broilers were involved in this detailed field study. This study demonstrated that replacement flocks can be reared and maintained in a Mycoplasma-free status in poultry houses with dirt floors. (Athens, Georgia, Research Laboratory)

Cooperative studies at the Connecticut Agricultural Experiment Station on several phases of work gave the following results:

Serology. Antigen for testing avian whole blood or serum for antibodies to Mycoplasma gallisepticum (PPLO), was produced under AIQ special license number 237 and sent to 64 research workers in 30 States, and 22 workers in 17 foreign countries. Because of the increased demand, this Station produced 520,000 doses during the past year, better than a 100% increase over the previous year (228,000 doses).

This laboratory has continued to survey the incidence of PPLO infection (M. gallisepticum) in poultry flocks in Connecticut in conjunction with the official Salmonella pullorum test and has found the majority of these flocks to react positively to our M. gallisepticum antigen, indicating a high rate of flock infection in Connecticut.

Control. Eight-week-old chicks were inoculated with live pathogenic M. gallisepticum and performance as regards growth rate, hatchability, fertility, egg production, mortality, etc., was compared to a control group. The immunized birds performed as well as the non-infected group, showing that, provided birds are vaccinated at the proper age and the infection is not complicated by other respiratory diseases, no harmful effects occur. At 23 weeks of age M. gallisepticum could not be isolated. In addition, upon challenge at 23 weeks with a pathogenic M. gallisepticum, no organisms could be isolated from the trachea or air sacs one week post inoculation. M. gallisepticum was readily isolated from a control group which was not previously immunized.

The inability to isolate M. gallisepticum after birds have recovered suggests that this procedure may eventually be adapted to an eradication program.

Isolation. A simple technique for isolation of PPLO from contaminated material has been devised using membrane filters (Millipore Filter Corp.) of 0.45 micron pore size. Material is suspended in broth and filtered using a syringe and Swinney adapter to hold the filter.

Using this technique M. gallisepticum has been isolated from feces and

litter from birds showing active symptoms of CRD. This procedure is well adapted to searching for PPLO which might be involved in diseases of unknown etiology. Present techniques of virology are inadequate since high levels of antibiotic are used which might inhibit growth of PPLO.

Nutrition and Physiology. In a basal medium consisting of tryptose, glucose and PPLO serum fraction (Difco) it was found that addition of Tris buffer and bovine hemaglobin increased the cell growth (Optical Density) by 30% which is of considerable importance in terms of producing antigen.

On blood agar plates, M. gallisepticum produced beta hemolysis of bovine blood agar. The hemolysis, however, does not appear to be related to pathogenicity since a strain of M. gallisepticum, which produces no disease in chickens and is unable to kill chick embryos, also produced beta-hemolytic zones.

In chemical studies of M. gallisepticum, several lipid fractions were identified. These were: saturated hydrocarbons, cholesterol, cholesterol esters, di- and triglycerides, free fatty acids, and 5 phospholipids. Using radioactive C^{14} oleic acid and P^{32} orthophosphate as tracers, it was shown that cholesterol esters, di- and triglycerides and phospholipids were synthesized from simpler compounds. (Connecticut)

Cooperative research on chronic respiratory disease at the Delaware Experiment Station during the year 1962-63, has been concerned with further studies on problems involved in the treatment of hatching eggs for the control of PPLO, an evaluation of various hatching egg treatments for PPLO control in broilers and the maintenance and serological study of the progeny of PPLO infected and free parents.

Attempts to provide a visual method of estimating the amount of antibiotic that enters an egg based on the previous observation that food dye will show up on candling soon after treatment have been made. There was good correlation between amount of dye and antibiotic content in white shelled eggs. However, with brown shelled eggs, detection of the dye was more difficult. The possibility exists of using this dye indicator system to eliminate eggs containing too little antibiotic in critical work aimed at eliminating PPLO.

Treatments of the egg shell with dilute acid to accomplish removal of the cuticle resulted in an enhancement of antibiotic absorption and bacterial contamination. On the other hand, shell thickness appeared to have no influence on drug absorption.

Work with bacterial contamination of eggs during the treatment process has established that bacterial entrance into an egg is enhanced in a similar fashion to drug absorption. The use of Hyamine 3500 was effective in controlling bacterial contamination with a Pseudomonas sp. and fungal contamination with an Aspergillus sp.

The antibiotic, Tylosin, was not effective in eliminating PPLO infection when administered at a 3 gram per gallon level for the first 5 days of life.

Several methods of treating hatching eggs and young chicks for the prevention of PPLO infection were without benefit when used on eggs and chicks from commercial sources in Delmarva. The level of PPLO infection was apparently absent or low in these experiments and this may represent a common occurrence under field conditions. This questions the need for routine preventative medication in broiler flocks.

High levels of chlortetracycline (1200 gms. per ton of potentiated feed) significantly lowered the number of birds reacting positively to the PPLO plate test and greatly reduced air sac lesions in birds experimentally infected with PPLO, E. coli, and infectious bronchitis virus.

A small flock of White Rock chickens has been maintained serologically free of PPLO for 40 weeks. They are being housed in an area near birds that are serologically positive. Under these conditions, the PPLO free flock averaged 7 eggs per month per bird more than those birds that are serologically positive. (Delaware)

Results of cooperative studies at the Georgia Agricultural Experiment Station tend to affirm that "PPLO-free" broiler chicks are able to withstand field (natural) exposure to CRD during the growing period and come through with a low incidence of air sac condemnation. This, however, cannot be assumed as a panacea for the problems of the broiler industry. Certain paramount responsibilities rest upon the broiler producer, viz:

1) There is no substitute for careful and well-planned management programs. The use of so-called "PPLO-free" chicks is no license to regress into poor and irresponsible management. With the investment in this breeding stock behind them, it behooves the hatchery organization to place these chicks with their best growers.

2) Vaccination programs normally used with PPLO-infected broiler chicks must be re-evaluated in terms of the "PPLO-free" chick's response.

3) Since "PPLO-free" breeding flocks are raised and maintained under more rigid control and management programs than normally followed for commercial broilers, the birds may not become naturally exposed to various "field" strains of infectious bronchitis virus. Susceptibility of their progeny to field strains must, therefore, be taken into consideration when setting up a vaccination program. (Georgia)

Cooperative research at the Massachusetts Agricultural Experiment Station yielded the following:

Properties of the agent (viability studies). The viability of 2 strains of Mycoplasma gallisepticum, virulent Hy and modified virulent, Adler-S6, is

influenced by the following factors: concentration of organism, temperature of storage, and the nature and quantity of suspending medium. The materials seeded with pleuropneumonialike organism (PPLO) included the following: PPLO broth, Grumbles' media (5 carbohydrates), PPLO agar, saline, chicken serum, egg albumen, egg yolk, infertile eggs, chicken muscle, cloth, chicken feces, and feather meal. The viability of the PPLO in the various test materials is given as a range at the different temperatures of storage: 37 C, 1 day to 45 weeks; 20 C, 1 day to 7 weeks; 6 C, 1 day to 17 weeks; and -20 C, less than 1 week to 78 weeks.

Transmission. A group of 16-month-old asymptomatic hens that had experienced a natural outbreak of CRD prior to 3 months of age transmitted the disease to susceptible yearling hens by cohabitation; that is, direct contact transmission. The period of exposure was limited to 65 days, the duration of the experiment.

Serology and Immunity. Yearling hens that have undergone a natural outbreak of CRD at an early age manifested definite resistance to challenge inoculation with a pathogenic PPLO; whereas, susceptible controls exhibited typical signs and lesions of the disease. Varying levels of CRD agglutinins may be manifested in progeny of positive parent stock, and may persist for as long as 18 days post-hatching. This parental agglutinin test may have some diagnostic significance in detecting CRD-infected flocks.

Response of CRD to medication. The performance of CRD-infected chickens following medication with high levels of Tylan in the water or Tylan injectable was superior to that of the infected-nontreated birds.

Control and eradication. CRD-free stock can be reproduced and maintained if adequate sanitation and management practices are observed. The majority of negative premises continue to remain negative year after year. Some flocks have remained negative for 4 or 5 successive years. There is an increasing interest on the part of flock owners in CRD-free progeny. (Massachusetts)

In cooperative studies at the University of Minnesota, a whole blood plate antigen for the testing of chickens for M. gallisepticum was developed. This antigen has been used to test 51,000 chickens in 29 flocks. Five of these flocks contained reactor birds with an average incidence of infection in these flocks of 579.

Several criteria have been used to classify avian Mycoplasma strains. Growth-inhibition by specific hyperimmune sera, hemagglutination activity, hemolytic activity and carbohydrate fermentation were used to analyze 62 avian Mycoplasma isolates. Thirty-six of these have been shown to be Mycoplasma gallisepticum.

The incidence of airsacculitis in day-old turkey poults was determined in 1197 poults. The average incidence was 24%. In an attempt to locate "Mycoplasma free" poults, several lots of poults from different hatcheries

were examined. A flock of Jersey Buffs was found to be free of detectable Mycoplasma. Poults from this flock were raised in isolation for experimental studies. In addition Tylan(R) was used in an attempt to produce "Airsacculitis free" turkey poults. Three poults of 557 examined from Tylan dipped eggs have shown airsacculitis, whereas 12 of 102 poults examined in the undipped controls had airsacculitis. This method of reducing airsacculitis will be further evaluated during the coming year.

Field investigations on 17 clinical outbreaks of infectious sinusitis were conducted. Contact with infected chicken flocks or other fowl and an outbreak in a turkey breeder flock were thought to be the possible source of the infection in some of the flocks.

Studies were continued on the effect of environmental conditions on artificial Mycoplasma gallisepticum infections. Two experiments were conducted with half of the birds held under what were felt to be near ideal temperatures, while the other half were held at lower temperatures. In both experiments the condemnation rate was higher in the groups held at the higher temperature, but the feed efficiency was lower under the cold environment so that the possible net profit would be lower.

Air samples were taken in hatcheries and on turkey farms in an attempt to quantitatively determine the microflora present. Large numbers of Staphylococcal sp., Bacillus sp., Alkaligenes sp., Proteus sp., Pseudomonas sp., E. coli, and E. freundi were found. The significance of these findings is unknown at the present time. Attempts are being made to correlate these findings with the organisms isolated from the air sacs of birds living in the same buildings.

The experimental control program on infectious sinusitis was continued on the basis of a 100% test of all turkey breeder flocks in the State and a flock inspection program of selected flocks in every hatchery. All indications are that infectious sinusitis continues to be held at a very low level in the turkey breeder flocks and their progeny. (Minnesota)

In cooperative studies conducted at the New York Agricultural Experiment Station, these results were obtained:

The presence of air sac lesions and the isolation of PPLO not M. gallisepticum from day old poults indicates a causal relationship. Furthermore, this evidence points to the egg transmissibility of the turkey strains of PPLO. The persistence of these organisms and lesions for many weeks in growing turkeys has been noted. Although this infection has not been associated with sinusitis in the birds studied, the air sac lesions may be confused with Mycoplasma gallisepticum infection. The serums of these turkeys do not agglutinate M. gallisepticum antigen. The attempt will be made to find poults free from this infection to initiate pathogenicity studies.

Dipping of eggs infected with Mycoplasma gallisepticum in solutions of Tylosin resulted in the hatching of non-infected chicks (with one exception) as proven by cultural methods. Such chicks when grown to maturity continued to be serologically negative (with exception of one group from eggs dipped in Erythromycin) when the agglutination test was applied. In contrast, non-dipped eggs produced infected chicks demonstrated by culture methods and persisted as serologically positive to maturity. A second generation of chickens hatched from eggs produced by dipped egg and un-dipped egg derived hens, were all serologically negative. It is clear that clean stock can be derived from infected dams by the egg dipping technique. It is equally clear that serologically positive dams need not necessarily produce infected progeny.

Inoculation of young chickens intra air sac with virulent Mycoplasma gallisepticum protected the birds completely from transmitting the organisms through the egg when the same birds were challenged at maturity. The degree of protection varied directly with the virulence of the immunizing cultures. Immunizing cultures of moderate to low pathogenicity induced a significant degree of resistance to later challenge but the protection was not complete.

Attempts are being made to determine whether serologically positive (M. gallisepticum antigen) birds can transmit M. gallisepticum to susceptible chickens by contact. Also experiments are in progress to determine whether the presence of cultivable M. gallisepticum from tracheal swabs is a better criterion for detection of spreaders. Tentative results indicate that contact transmission of M. gallisepticum need not occur even though serologically positive birds (from in ovo infection) and culturally negative are in contact with completely susceptible birds. On the other hand, in one trial the opposite result was obtained although in this case the serologically positive birds had been infected 16 weeks prior to contact with susceptibles. It appears that the efficiency of cultivation techniques for isolation of M. gallisepticum from tracheal swabs leaves much to be desired. (New York)

Cooperative studies at the North Carolina Agricultural Experiment Station have been made on quantitative and qualitative blood studies in relation to disease susceptibility of chickens experimentally infected with respiratory disease viruses, with special reference to in vitro and in vivo determinations of anti-E. coli activity.

Plasma influence on the bird's total resistance to E. coli appears to be relatively small since in vivo tests involving homologous and heterologous treatment of E. coli organisms and subsequent bird inoculations offered only slight differences in protection to the inoculated birds. (North Carolina)

Cooperative studies have been conducted at the Texas Agricultural Experiment Station with the following results:

Infectious Sinusitis Eradication Program. One hundred-eighty (180) breeding flocks, representing 217,017 birds and approximately 80 percent of the turkey

breeding stock in Texas, were enrolled in the 1962 program. Random sample (10 per cent) testing was used. Five M. gallisepticum infected flocks were identified and marketed.

Chronic respiratory disease control. A small replacement breeder flock was hatched from eggs produced by known M. gallisepticum infected breeders. The eggs were dipped in chilled antibiotic solution prior to incubation. The replacement flock is now in peak production and apparently free of CRD.

Attempts are being made to bring a large commercial breeder flock (20,000 birds) into production free of CRD. The birds, progeny of M. gallisepticum free breeders, were free of infection when 10 weeks of age.

M. gallisepticum antigen. Further refinement of M. gallisepticum antigen and antigen production techniques were made.

PPLO serotyping. Preliminary studies on the use of a cytopatnogenic effect-inhibition (CPE-I) technique to serotype PPLO in tissue culture system were made.

Wildlife reservoirs of M. gallisepticum. Thirteen wild turkeys were serologically examined for evidence of M. gallisepticum infection and were found to be negative. (Texas)

Cooperative work at the Virginia Agricultural Experiment Station on the CRD complex was directed toward the defense of the chicken to one of the most commonly found bacteria in air sac infection, E. coli. A unique approach was employed to arrive at the determinations made - a continuous-flow intravenous inoculation of the bacterial culture over a 24-hour period, or throughout a 14-day period. The pathogenic bacteria were given at the rate of 10 million to 1 billion organisms daily for 1 - 14 days. A separate hookup for taking electrocardiograms was also maintained.

From 8 minutes to 6 hours or 1 day after the beginning of continuous inoculation the blood cultures were maintained at about 100-300 bacteria per ml. The lymphocyte counts steadily fell until 12-24 hours when they virtually ceased to be present till the 3rd day when they slowly increased in number. The heterophile blood counts reached a low level at about 1 hour after which they rose to from 30,000 or 60,000 in birds that resisted pericarditis. The rise in heterophile counts preceded a period of complete clearing of the blood of bacteria which occurred between 6 and 24 hours after the beginning of bacteremia and continued for 6 hours to 3 days. In birds which developed pericarditis the period of complete clearing was very short and the rise in heterophiles to 15,000 per ml was of short duration. Eventually the high heterophile counts of birds which did not develop pericarditis fell and the period of complete clearing of the blood came to an end. After this fall the blood contained from 500-5,000 bacteria per ml and the heterophile count rose to very high levels. After the 7th day the blood culture level fell in association with a rise in the lymphocytes count.

Body temperature tended to remain normal but often rose to 108°F just before death. Plasma albumin often became drastically reduced as exposure progressed. Gamma globulin rose during the course of exposure.

By means of an electrocardiogram it was possible to detect the onset of pericarditis.

The reaction of the chicken to continuous intravenous exposure seemed to be considerably different from the reaction to a single dose. It appears that pericarditis must occur early in the period of exposure or not at all. (Virginia)

Cooperative work at the Wisconsin Agricultural Experiment Station has been directed to studies on measuring the environment of turkeys raised in confinement. Three experimental flocks of turkeys have been reared in the Meteoropathology Building. The primary emphasis has been placed on methods for study of the influence of environment on respiratory diseases of turkeys.

Temperature, humidity, atmospheric dust, ammonia and carbon dioxide, light intensity, sound, bird activity, water consumption, feed consumption, bacterial content of the air and litter, moisture content of the litter, are some of the factors being measured. Birds are regularly removed and sampled for disease organisms. Mycoplasma, staphylococcus and unidentified viruses have been recovered. The relationship of mycoplasma isolated, which is not of the S6 type, to N type is under study. The presence of this organism appears to be causally related to clinical air sacculitis. Techniques have been perfected for the recovery of mycoplasma from aerosols. The half life of the organism in the air and the biology of transmission is being studied. (Wisconsin) (ADP a5-17) (ADP a5-21)

C. Newcastle Disease

In cooperation with the Wisconsin Agricultural Experiment Station, current research on Newcastle disease virus was evaluated at an International Symposium held in Madison July 15, 16 and 17, 1963. Much of the planning for this event was undertaken by the Wisconsin staff. Important questions unresolved are interepizootic persistence, mechanisms of transmission, nature of virulence, evolution of new antigenic strains.

Previously we have shown that chicken virulence and time of embryo death can be related. It now appears that plaque morphology may also be related to virulence. Virus (Herts strain) of the large clear plaque type induces severe hemorrhage in embryos and rapid death, whereas virus of the small plaque type isolated from the same strain fails to induce hemorrhage and kills after a prolonged incubation. Virulence characteristics of all plaque types are under study.

Other work concerns development of avian cell lines, evaluation of serological methods for antigenic analysis of strain differences, and quantitative studies of NDV aerosols. (Wisconsin)

In cooperative work at the University of Maine, the evaluation of killed Newcastle disease vaccine in chickens is in progress. A specific pathogen free (SPF) program has been conducted on broiler and breeder flocks during the past year - 1962-63. A rigid set of standards for isolation and husbandry are required to conform to the program. A total of 20 SPF breeding flocks, comprising 70,960 birds, have been on the program. Approximately 142,654 samples have been tested for PPLO. A method of mass testing (tube agglutination) has been developed.

A total of 65 SPF broiler flocks, totaling 1,502,862 birds, with an average weight of 3.78 pounds at 9 weeks of age, have been processed. Forty-six flocks have been free of infectious bronchitis; 34 of PPLO and 62 of Newcastle disease.

Approximately 4 million doses of dead Newcastle disease vaccine have been used in the State of Maine this year with no evidence of "breaks." (Maine) (ADP a5-18)

Work at the Institute of Veterinary Research, Pulawy, Poland, under a PL 480 grant, has shown that thirty passages of virulent NCD virus through chickens infested with roundworms, and through chickens previously infected with fowl cholera, failed to change the pathogenic properties of the virus.

D. Bluecomb in Turkeys

Contract investigations at the University of Minnesota show that bluecomb disease continues as one of the serious disease problems of turkeys of all ages. The enterovirus that has been consistently isolated from the intestinal tract of poults involved in bluecomb disease did not reproduce the bluecomb syndrome when given to 1-day and week old turkey poults.

Poults challenged three days after such an oral feeding of the virus did not have any immunity when exposed to intestinal material.

Tissue culture studies with chicken and turkey primary cell cultures indicated the enterovirus did not produce cytopathogenic effects in these cultures.

Immunity studies are continuing to determine what relationship the entervirus has with the bluecomb disease and if it will produce any protection against the disease. (Minnesota) (ADP a5-19(C)

E. Avian Leukosis

Studies on this problem under cooperative agreement with the Regional

Poultry Research Laboratory, USDA, East Lansing, Michigan, will be reported by the Poultry Research Branch of the Animal Husbandry Research Division.

Basic studies on this problem conducted at Cornell University under cooperative agreement, were directed to attempts to substitute cytopathogenic viruses for Rous Sarcoma virus in the resistance inducing factor (RIF) test. This work is best presented as follows:

Rubin has shown that chick embryo fibroblast cultures infected with Resistance Inducing Factor (RIF-virus) did not support the growth of Rous sarcoma virus (RSV). Experiments were designed to demonstrate whether inhibition would also occur when RIF-infected cells were challenged with other cytopathogenic viruses.

Fibroblast cultures were prepared from chick embryos known to be free of RIF-virus. The cells were divided into two lots; one maintained serially as a control line, the other inoculated with RIF-virus and carried serially as an infected line. When preliminary trials indicated that the RIF-infected cell line had developed resistance to RSV, parallel titrations with RSV, fourteen avian entero-viruses, Newcastle disease virus, infectious laryngotracheitis virus, and canine distemper virus were done in both RIF-free and RIF-infected cultures. Two trials using a microscopic-plaque technique and one trial using gross-plaque techniques were done. Results indicated that RIF-infected fibroblasts, while resistant to RSV proliferation supported the growth of the other viruses and underwent the cytopathic changes produced by them. (Cornell) (ADP a5-22)

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AREA 6 - INFECTIOUS AND NON-INFECTIOUS DISEASES OF FUR ANIMALS, INCLUDING RABBITS

Problem. In the raising of fur animals in captivity, such as rabbits, chinchillas, mink, and foxes, disease problems incidental to the confinement of such animals are encountered. These include viral, bacterial, parasitic, mycotic, nutritional, and hereditary diseases. The enteric disease-complex causes great mortality in commercial rabbit production. It destroys whole litters and commonly attacks all susceptible rabbits on a farm. The respiratory disease-complex, perhaps, is second as a cause of mortality. In severe outbreaks over 50 percent of adult animals may die. These two diseases cause great economic loss to the rabbit industry, which produces an estimated 50 million pounds of meat annually and millions of dollars worth of rabbits for experimental purposes. Virus diseases of mink cause the greatest loss to the 7,000 mink ranchers now producing more than 5 million pelts annually valued in excess of $100 million.

USDA PROGRAM

The Department has a continuing long-term program involving microbiologists and veterinarians engaged in both basic studies and the application of known principles to the solution of infectious and non-infectious diseases of fur animals, including rabbits. Research is being conducted on the following diseases at the designated locations.

The Federal scientific effort devoted to research in this area totals 3 professional man-years. This effort is applied as follows:

Enteric Disease Complex of Rabbits, 0.5 at the U. S. Rabbit Experiment Station, Fontana, California.

Respiratory Disease-Complex of Rabbits, 0.5 at the U. S. Rabbit Experiment Station, Fontana, California.

Coordinated Field and Laboratory Studies, 1.0 at the U. S. Fur Animal Disease Research Laboratory, Pullman, Washington.

Transmission of Infectious Diseases by Helminths, 1.0 at the U. S. Fur Animal Disease Research Station, Pullman, Washington.

REPORT OF PROGRESS FOR USDA

A. Enteric Disease-Complex of Rabbits

The stress of rapid rebreeding of the doe and early weaning of the fryers had little or no effect on enteritis mortality. An additional period of time was required for some of the fryers to make the average weaned weight commonly associated with the 8-weeks weaning schedule.

Ethopabate, a water soluble coccidiostat that has been reported as being very effective in controlling coccidiosis in poultry, was found to have no effect in controlling liver-type coccidiosis in the rabbit.

A study was made of an outbreak of favus due to Trichophyton mentagrophytes in a herd of rabbits. Transmission was traced to field mice found in feed storage units and infected with the same organism. Oral medication with felvicin was found to be very effective in controlling the infection.

A study was made on bacterial contamination and shelf life of freshly eviscerated rabbit fryers. While no direct correlation could be made between bacterial population and spoilage, several factors appear to be beneficial in prolonging shelf life and lowering bacterial count. These factors were: thorough washing of rabbits during processing; using more than one man for killing, skinning, and eviscerating the rabbits, and holding the carcasses no longer than 1 hour in chill water. Prolonged immersion in chill tap water was found to have an adverse effect on bacterial population and duration of shelf life. (ADP a6-5)

B. Field and Laboratory Study of the Diseases of Fur Animals

1. Fox Encephalitis Virus. While plaque formation of fox encephalitis virus (infectious canine hepatitis) has been reported, comparison of plaques produced by various isolates has not been described. Plaques were observed 6 days after seeding. They increased in number until the 9th day, occasionally until the 12th day after inoculation. Wide range of variation in plaque size was observed with each of the isolates (American and Japanese). Difference in plaque morphology among isolates was not observed.

2. Hereditary Abnormal Leukocyte Granules in Mink. Anomalous granules and inclusions of mink leukocytes similar to those in Chediak-Higashi syndrome of man was observed. The hereditary pattern of these abnormalities was examined. The leukocyte abnormalities consisted of granules of variable size (0.5 to 1.0 micra) in neutrophils, monocytes, and lymphocytes. Eosinophils exhibited variation in size and shape of cytoplasmic granules. Of the 449 mink, 230 had abnormal leukocytes. All of these were of the Aleutian aa genotype. The remaining 219 had no abnormal cells and were non-Aleutian AA or Aa genotypes. These observations indicate abnormal leukocyte granulation in mink is a non sex-linked recessive character of Aleutian aa mink.

3. Chediak-Higashi Syndrome in Cattle. The panleukocytic anomaly which this Laboratory first reported in mink has also been found in the Washington State University herd of partial albino Hereford cattle. It is believed, in man, that this is a non sex-link simple homozygous recessive characteristic and that individuals with the syndrome (man, mink, and cattle) are unusually susceptible to bacterial disease. This is a major finding, perhaps making for the first time a marker (white blood cell granules and coat color) for susceptibility to disease. Studies are under way to quantitate the susceptibility.

4. The Use of the Iodine Agglutination Test (IAT) for the Diagnosis of Aleutian Disease (AD). The results of this test for the diagnosis of subclinical Aleutian disease has been very good. The losses on cooperating ranches were reduced by approximately one half during 1962. The test is the only control for the disease at the present time, and from these findings, the test has an accuracy of about 90 to 95 per cent.

5. The Familial Occurrence of Aleutian Disease. Thirty-one families of mink on a ranch where spontaneous Aleutian disease was enzootic were tested for hypergammaglobulinemia. A statistically significant higher prevalence in offspring from affected dams was found. These results suggest that a particulate agent may cause a disease which is familial in occurrence. Genetic relationships and similarities of occurrence of Aleutian disease and certain connective tissue diseases of man are being considered.

6. Experimental Hypergammaglobulinemia (AD) in Mink. Hypergammaglobulinemia in mink was produced by the injection of crude tissue suspensions from mink with spontaneous Aleutian disease. The initiating factor was found to be resistant to 0.3 per cent formalin for 2 weeks but not 40 weeks at 5°C. Foreign antigens as well as formalinized normal mink tissue from homologous and heterologous genotypes did not cause a detectable change in the serum protein values.

Mink homozygous recessive for the Aleutian gene were found to be significantly more susceptible to the experimental disease.

7. The Effect of an Anti-Inflammatory Drug on the Progression of the Aleutian Disease. Long term treatment with an anti-inflammatory drug (dexamethasone) has doubled the incidence of spontaneous AD in a large group of mink on an affected ranch.

8. Hypergammaglobulinemia (AD) in Mink Initiated by a Cell-Free Filtrate. It would appear that the cause of hypergammaglobulinemia in mink is a filterable substance which can be almost completely removed from aqueous suspension by ultracentrifugation at 95,000 g for 1 hour.

The finding of a filterable, cell-free substance which initiates changes in mink that resemble certain connective tissue diseases in man raises speculation as to possible similar mechanisms involved in these human diseases.

9. The Use of Attenuated Feline Panleukopenia Virus for the Immunization of Mink Against Mink Virus Enteritis. Data obtained indicate resistance to virulent MVE in mink can be induced by subcutaneous injection, and to a lesser extent by the oral administration of a variant of panleukopenia virus. In this trial, the onset of immunity occurred at 3 days following injection. This rapid onset is important in the control of outbreaks. Using the evidence obtained from the field, it appears that the onset of immunity following vaccination with inactivated MVE virus is between 10 and 14 days.

10. Use of the Attenuated Feline Panleukopenia Virus in Neutralization Tests. Neutralization tests were conducted using the attenuated isolate of feline panleukopenia. The virus was tested against sera from animals immunized with virulent panleukopenia and mink virus enteritis virus. Significant neutralization was obtained using serum from panleukopenia immune ferrets and mink, panleukopenia hyperimmune cats, and mink virus enteritis immune mink. No neutralization of the virus occurred with normal ferret, mink, and cat sera.

11. Further Note on the Relationship of Mink Virus Enteritis to Feline Panleukopenia. Inactivated mink virus enteritis vaccines containing an adjuvant protected experimental cats against challenge with virulent panleukopenia virus. Field use of tnese vaccines supported the laboratory findings. (ADP a6-7)

C. Studies on the Persistence and Transmission of Viral and Rickettsial Diseases in Helminths Associated with Diseases of Fur Animals.

Transmission of a rickettsial disease from a warm blooded definitive host (dog) to an adult trematode (Troglotrema salmincola) was demonstrated.

Studies on the adult trematode in warm blooded definitive hosts reveal that salmon poisoning flukes are capable of expelling eggs over a very long period of time (up to a year).

In studies on the redial and cercarial stages it was not possible to demonstrate the persistence and transfer of the rickettsial agent from the redia to the cercaria beyond the second clutch of cercaria under laboratory conditions.

It has been demonstrated that cercaria of Troglotrema salmincola can be killed by the use of ultrasonics at one megacycle frequency in running water.

The finding of metacercaria persisting for at least 4 years, containing the infectious rickettsial agent, has been confirmed. Studies of the geographical distribution of this parasite reveal not only may salmon be found infected with rickettsia containing metacercaria as far north as the 59th parallel, but that the salmon obtained from the Sacramento river also

contained rickettsia-bearing metacercaria. This extends the known geographic distribution of salmon poisoning from the 59th parallel north to at least as far as San Francisco to the south.

It has been found that copper sulfate may be used efficiently as a molluscacide in small tributaries feeding hatcheries in which this parasite is indigenous.

The use of complement-fixation as a serological technique for the study of salmon poisoning has been shown to be a useful laboratory tool. (ADP a6-8)

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AREA NO. 7 - MISCELLANEOUS INFECTIOUS AND NON-INFECTIOUS DISEASES OF ANIMALS

Problem. Included in this area of research are diseases such as vesicular stomatitis, which affects cattle, horses, swine, and man; poisoning by various plants, which differ in toxicity according to local conditions, and affect different species of animals in various ways; agricultural chemicals, such as herbicides and pesticides, which may produce poisoning in animals, especially if not properly used, and may also leave dangerous residues in the soil, feed, or animal body; tumors, including cancer, which affect all species of animals; bloat, a common, serious condition in cattle and sheep; and potential dangers of "fall-out" from nuclear testing or attack. Investigations of these diverse hazards to livestock and poultry require modern techniques as well as fundamental approaches through chemistry, pathology, physics, physiology, and other scientific disciplines. The problems are so complex, diverse, and numerous that it has been impossible to more than scratch the surface in probing for basic knowledge required for protection of the nation's livestock and poultry populations.

USDA PROGRAM

The Department has a continuous long-term program involving biochemists, microbiologists, pathologists, physicists, and veterinarians engaged in both basic studies and the application of known principles to the solution of miscellaneous infectious and non-infectious diseases of animals. Research is being conducted at the designated locations.

The Federal scientific effort devoted to research in this area totals 21.0 professional man-years. This effort is divided among sub-headings as follows:

Incidence and Pathology of Tumors 1.0 at the National Animal Disease Laboratory, Ames, Iowa. A grant of PL 480 funds equivalent to $51,383 has been placed with the Veterinary Faculty, Ankara University, Ankara, Turkey, on etiologic investigation of bovine urinary bladder tumors due to enzootic bovine hematuria in Turkey and its relation to bovine papilloma agent.

Vesicular Stomatitis 1.0 at the National Animal Disease Laboratory, Ames, Iowa.

Components of Normal and Immune Serum 1.0 at the National Animal Disease Laboratory, Ames, Iowa.

Bloat in Ruminants 4.5 at the National Animal Disease Laboratory, Ames, Iowa, and through cooperative agreements with the California, Maryland, and Mississippi Agricultural Experiment Stations and with the New York State Veterinary College.

Preparedness for Diagnosis of Foreign Animal Diseases 2.5 at the Plum Island Animal Disease Laboratory, Plum Island, New York.

Toxicology and Pathology Related to Insecticides 2.5 at the ADP Field Station, Toxicology Laboratory, Kerrville, Texas, in cooperation with the Entomology Research Division.

Biochemical Effects of Agricultural Chemicals 0.9 at the ADP Field Station, Toxicology Laboratory, Kerrville, Texas, and through a cooperative agreement with the Stephen F. Austin College at Nacogdoches, Texas.

Detoxication Mechanisms in Cattle and Sheep 0.5 at the ADP Field Station, Toxicology Laboratory, Kerrville, Texas.

Cytological Responses to Antiparasitic and Other Agricultural Chemicals 0.5 at the ADP Field Station, Toxicology Laboratory, Kerrville, Texas.

Poisoning by Plants 1.1 at the Logan, Utah, Field Station, through formal cooperation with the Utah Agricultural Experiment Station, and informal cooperation with the U.S. Plant, Soil and Nutrition Laboratory of the Soil and Water Conservation Service, Ithaca, New York. A PL 480 grant of $56,746 was placed with the Instituto Biologico, Sao Paulo, Brazil, on The Study of Plants of the State of Sao Paulo poisoning to domestic animals.

Toxicity of Herbicides and Herbicide-Treated Plants for Domestic Animals 1.0 at the Logan, Utah, field station, with informal cooperation with the Utah Agricultural Experiment Station and the Crops Protection Branch of the Crops Research Division at Logan, Utah.

Alleviators and Diagnostic Tests for Plant Poisoning 1.0 at the Logan, Utah, field station through informal cooperation with the Utah State University, the Crops Research Division and the Forest Service.

The Susceptibility of Wild Animals to Foot-and-Mouth Disease 0.5 at the Plum Island Animal Disease Laboratory, Plum Island, New York.

Mycotic Diseases of Domestic Animals 3.0 at the National Animal Disease Laboratory, Ames, Iowa.

REPORT OF PROGRESS FOR USDA AND COOPERATIVE PROGRAMS

A. Components of Normal and Immune Serum.

The research on bovine plasma acid glycoproteins was extended to include chemical analysis and identification of the components of the carbohydrate moieties which represented 22 per cent of the M-2 glycoprotein and 41 per cent orosomucoid. The content of sialic acids of the two proteins was found to be 9.5 and 16.2 percent respectively. The sialic acids appeared to be a mixture of N-acetylneuramine and N-glycolylnuraminic acids with a slight

predominance of the latter. The predominating, if not the sole, hexosamine type appeared to be glucosamine and constituted 6.4 and 11.8 per cent respectively of the two glycoproteins.

With the exception of 0.4 per cent fucose and 0.9 per cent uronic acids in orosomucoid, the only other carbohydrates detected were galactose and mannose. These were present to the extent of 4.0 and 3.7 per cent respectively in M-2 glycoprotein and 0.8 and 7.3 per cent respectively in orosomucoid. (ADP a7-14(Rev.)

B. Bloat in Ruminants.

In cooperation with the California Agricultural Experiment Station's Animal Husbandry Department at Davis, studies have continued on the relationship between concentrations of substances in the rumen ingesta and the rate of salivary flow and composition in cattle. In view of previous work on this project, which established the importance of saliva in legume bloat, it was thought desirable to investigate whether substances in the rumen, acting either directly on receptors within the rumino-reticular walls or on the salivary glands via the blood stream following absorption from the rumen, regulate the amount and composition of saliva entering the rumen. Such a postulated system could provide a sensitive "feed-back control mechanism" for regulating the composition of rumen ingesta. A failure in such a mechanism could be an important factor in the etiology of bloat.

In preliminary studies over the past year, two cattle have been prepared with esophageal and large ruminal fistulas. Salivary flow rates and composition were determined before and after introduction of water, acetic, or hydrochloric acid solutions into the empty rumen. The two individuals differed markedly in their response to these materials. Usually, intraruminal infusion of water produced a slight increase in salivary flow rates of both animals. Both acetic and hydrochloric acids caused a marked and sustained elevation of salivary flow in one animal but had no appreciable effect on the other. Possible causes for this individual difference are under investigation. Regardless of how produced, changes in salivary flow in both animals were associated with significant changes or lack of change in the concentration of several salivary constituents. At low flow rates, potassium and chloride ion concentrations increased, sodium ion concentration decreased. Phosphate ion concentration increased, and bicarbonate concentration remained unchanged with increasing salivary flow rates. Additional investigation of such relationships should provide insight into the mechanism of salivary synthesis and secretion in cattle. (California)

At Cornell University, the work of the past year, carried out on cattle and calves, was in continuation of the work of the previous year, on quantitative studies on blood flow to major forestomach compartments and outflow of digesta from the first two of these. An external impedance-matching circuit for the electrical flow-sensing devices, which permitted daily assessment of

the status of chronically-implanted animals and which provided for better interpretation of records, was described. Tandem pressure-sensing devices and fistula preparations of the 3rd and 4th stomach compartments, were combined with the above to study normal digestive processes and experimental indigestion conditions. (New York)

In cooperation with the Maryland Agricultural Experiment Station, the effects of certain sympathomimetic and parasympathomimetic drugs in combating and inducing bloat in sheep were determined. The results suggest that some components of legumes and other bloat diets produce bloat by inhibiting the parasympathetic and/or by increasing the activity of the sympathetic system. (Maryland)

At the Mississippi Agricultural Experiment Station, no grazing data or rumen samples were collected for steers grazing crimson clover during the spring of 1962. This was due to the loss of the very young clover stand which was killed by severe cold weather and extremely large amounts of rainfall during the months of January, February, and March. Due to the lack of crimson clover, the following report deals with work pertaining to Ladino clover bloat studies conducted in 1962. Several rumen samples were removed from bloaters and non-bloaters three times per week for a 2-week period. The samples were analyzed for microbial population by a staining technique and counted under a microscope, pH was determined for samples, and buffer capacity was taken by the addition of 10 N sulfuric acid to lower the pH down to 1. The technique used in this study showed no difference in encapsulation of bacteria in rumen content of bloaters and non-bloaters. The data from this study indicated that there was a higher pH of ruminal content of bloaters than there was for the non-bloaters. There appeared to be no difference in the buffering capacity of rumen content for either bloaters or non-bloaters.

During the 1963 spring grazing season, 10 calves were grazed on crimson clover for about 25 days. Only 2 calves bloated a total of 20 times out of a possible 100 times and there was no severity greater than moderate bloat. The two "bloaters" and 2 steers which did not bloat, were used for obtaining samples of rumen content. The rumen content will be analyzed for volatile fatty acids, calcium, phosphorus, and potassium. (Mississippi)

Cooperative work was continued with the Wisconsin Agricultural Experiment Station to understand better the processes occurring in bloat, experiments being directed toward identifying and measuring 1) some early enzymatic reactions in the rumen, and 2) effects of some factors in the rumen on specific enzymatic reactions. Experiments in controlling bloat have also been completed.

Pectin dissolved in water, centrifuged and strained ruminal fluids formed firm gels when calcium chloride and sufficient pectin methyl esterase (PME) were present. Addition of alkyl aryl sulfonate to the pectin solutions prevented the formation of pectin gel by the enzyme.

A 96% reduction in the incidence of experimental bloat in 24 dairy cows fed fresh legume crop was obtained by feeding an enzyme inhibitor, alkyl aryl sulfonate prepared for controlled release in the rumen.

A model system of gelling by PME was developed with pectin, calcium chloride and PME in phosphate buffer. An inhibitor of PME activity was found in strained ruminal fluid and in the precipitate after centrifuging this strained ruminal fluid. A reversal of pectin gelling was observed when a low concentration of enzyme and of pectin was used.

Further studies of pectin gelation in presence of calcium chloride and in ruminal fluids by PME prepared in phosphate buffer and sodium chloride have been completed. The addition of alkyl aryl sulfonate, ultrawet K Dense and ultrawet 60L each to pectin, PME and calcium chloride inhibited the formation of a gel. This detergent inhibition was reduced or overcome by the addition of either soybean protein or casein to the pectin gelling system. When the reaction of pectin and PME was carried out in centrifuged ruminal fluids a gel formed without the addition of calcium chloride. The presence of a heat labile factor and a "gel forming" cofactor were found in centrifuged ruminal fluid. The heat labile component caused a slow reversal or liquification of pectin gelation and reduced the firmness of the gel formed. The ruminal fluid has sufficient "gel forming" cofactor to produce a pectin gel that was comparable to that formed in water by the gelling system containing 0.008M calcium chloride. Verxite-treated calcium chloride had no effect on pectin gelation.

Finally the starch-digesting activity in some natural sources and in commercial standards was determined by inoculating a corn starch substrate in petri dishes. The clear zones obtained after flooding the surface with I_2-KI solution were measured. The greatest starch-digesting activity at 24 hours was present in human saliva, alfalfa extracts and, of course, in the commercial standards. The incubation for the enzymatic activity can be reduced from 24 to as short as 6 hours and still obtain good measurable zones with the commercial standards. (Wisconsin) (ADP a7-15)

C. Preparedness for Laboratory Assistance in the Diagnosis of Animal Diseases.

Teschen disease virus, frequently a cause of paralysis and other nervous signs in pigs, appears to be a member of a group of viruses similar in many respects but differing in ability to affect nervous tissue. Comparative studies conducted at PIADL show that some viruses recovered from pigs in the United States probably belong to the group related to Teschen disease virus but do not share the capacity for damage to nerve tissue. Precise methods are required to differentiate between the exotic disease virus and those submitted for study from laboratories in the United States. (ADP a7-16)

D. Toxicology and Pathology Related to Insecticides.

The development of a colorimetric analytical method for animal tissues containing 2,4-D was successful, largely because of the use of Carbon 14 labeled 2,4-D which could be determined with radiation detection instruments at each step of the developing technique. The analytical method which resulted is capable of detecting 0.05 parts per million of 2,4-D in animal tissue samples weighing 25 grams.

Carbon 14 labeled 2,4,5-T was ordered but not received in time to be utilized during FY 1963. It will be used to develop analytical methods and to study detoxication mechanisms. (ADP a7-12(Rev.)

Thirty-two insecticides, most of them currently under exploration by the Entomology Research Division, were studied during the fiscal year. Those identifiable only under a number have no meaning in this summary, but the studies conducted furnish guidelines for decisions for further development of them.

Co-ral and arsenic did not appear to show potentiation in cattle and calves when used on the same day or within one or two days of one another. The study was conducted to show the safety of using both compounds on cattle imported from Mexico.

Sprays of toxaphene following Co-ral sprayings seemed to reactivate the Co-ral deposits, leading to mild Co-ral poisoning in cattle and calves so treated.

Three chemosterilants (compounds that might be used to produce sexual sterility in insects), apholate, aphoxide and methaphoxide were found to be highly cumulative in effect. Although some studies using low-level feeding are still current and the animals still alive, every dosage thus far tried has ultimately killed every sheep treated, in some cases after 1 year or more of exposure during which no observable illness occurred.

Three fungicides and thirteen herbicides were studied in cattle and sheep; one fungicide was studied in chickens. Generally, except for the mercurial fungicides, massive dosages repeated on several days were required to produce poisoning. Four herbicides, simazine, atrazine, bandane and promazine were found to afflict the nervous system. With bandane, a yearling steer died of a cerebral and medullary hemorrhage after showing various degrees of paralysis and other neurological symptomatology. (ADP a7-23)

E. Biochemical Effects of Agricultural Chemicals.

The ordinary feeding of vitamin A to cattle was found to increase their susceptibility to poisoning by Co-ral, particularly when they were also given phenothiazine drenches for internal parasite control.

When impurities appeared in Co-ral, as they did during the fall of 1962, the toxicity was even greater. Contaminated Co-ral, such as was credited with causing losses of cattle exceeding $750,000 in value, could not be shown to produce poisoning in Kerrville cattle unless vitamin A or phenothiazine were also used.

These initial studies need to be followed with others to determine whether the effect is limited to Co-ral or also follows the use of other insecticides.

More important than the increased incidence of poisoning when the vitamin A and phenothiazine are present is the type of poisoning produced. Whereas Co-ral can poison and kill any animal, its activity normally is against an essential enzyme, cholinesterase. The animals that do not die rarely show any appreciable tissue changes. In the poisoning observed this year, there is marked tissue change. One of the changes noted was a necrosis (death) of muscle fibers, particularly of the thigh muscles that compose the "round" meat cuts. In the living animal the necrosis is apparent as a lameness, in the carcass as an area of "white muscle" showing clearly against the normal red.

Also important is the observation that severe weight losses occur in the absence of observable symptoms. The reason for the losses is not at all clear. The importance of weight losses instead of weight gains to the feeder of cattle needs no explanation.

Four important enzyme systems were affected by the vitamin A - phenothiazine - Co-ral combination. The significance of the effect is not yet clear.

Brahman cattle, and their crosses, were shown to be more susceptible to poisoning by Ciodrin and Compound 4072 than European breeds and their intercrosses. The blood enzyme, cholinesterase, was more readily inhibited in Brahmans and their crosses than in European breeds. With Compound 4072, the only chemical given both by mouth and as a spray, the susceptibility was greater in Brahmans by both routes, indicating that the peculiarities of the Brahman skin were secondary to the species difference. The increased susceptibility was most marked to the compound Ciodrin.

Work has continued on the particle size spectrometer with the Physics Department of the Stephen F. Austin State College of Nacogdoches, Texas. The theoretical scattering pattern for acoustic waves impinging on a sphere has been re-calculated and several minor errors corrected. A theoretical study of the change in sound velocity caused by impurities in gas has progressed to such a point that experimental verification is necessary. Since the change in velocity depends on particle size, it is believed that this may give a method of determining particle size in an aerosol or an emulsion. A detection circuit has been added to the Ionovac speaker which allows it to be used as an ultrasonic microphone up to 45 KC.

In cooperative work with the Chemistry Department of the Stephens F. Austin State College, the solubilities of potassium antimony tartrate have been determined at several different temperatures. Preliminary solubility studies have been made. These measurements involve the amount of material that will dissolve in water at a fixed temperature. Some data is available on the dissolving of barium chloride in a saturated solution of potassium antimony tartrate. (Texas) (ADP a7-18)

F. Detoxication Mechanisms in Cattle and Sheep.

Oximes, including 2-PAM chloride, DAM, and P_2S, were used to counteract poisoning by various organophosphorus compounds. The oximes are useful to cause a release of the enzyme (cholinesterase) inhibited by this group of pesticides. All three compounds were effective in mild or moderate poisoning. Their action is somewhat slow, therefore we found that the use of our old favorite, atrophine sulfate, was still required in severe poisoning to gain time for the oximes to work.

Sodium selenite, sodium selenate and d-alpha tocopheryl (Vitamin E) were found to be effective in several instances of organophosphorus poisoning in our research. One veterinary practitioner applied selenite and tocopheryl in his practice and reported excellent results. Although we are confident of the results observed, we need to explore the mechanism by which these two substances exert their beneficial effects.

Studies of lindane sheep dips revealed that improper physical formulations were being employed, permitting the first sheep dipped in a fresh vat to so deplete the fluid that the sheep dipped later were receiving dangerously small concentrations insofar as control of parasites such as the scabies mites are concerned. The excessive amounts taken out by the first sheep dipped undoubtedly caused them to develop tissue residues far in excess of those normally to be expected. Regulatory officials enlarged upon these studies, then removed lindane from the list of approved dips until proper formulations could be offered by the manufacturers.

Residues of 2,4-D in sheep fed the compound at a rate of 2 grams per head per day at Logan, Utah, were determined at Kerrville, using an analytical technique developed by the Kerrville staff. In sheep fed 30 daily doses, kidney, rumen, renal fat and body fat samples showed less apparent residue than did a control sheep. Muscle samples averaged less than 0.3 p.p.m., and liver samples less than 1.0 p.p.m. of 2,4-D. (ADP a7-19)

G. Cytological Responses to Antiparasitic and Other Agricultural Chemicals.

Five thousand slides for microscopic examination were prepared from tissues of 116 animals that died during various toxicological studies.

In animals killed by the insect chemosterilants apholate, aphoxide, and methaphoxide, cytological changes were most prominent in the organs engaged in formation of the white cells of the blood. The changes indicated a severe decrease in ability of the parent tissue to supply the needs of the animal. (ADP a7-20)

H. Poisoning by Plants

1. Cyclopian-Type Malformation in Lambs. The study of a congenital cyclopian-type malformation in lambs has been continued in cooperation with the National Animal Disease Laboratory, Ames, Iowa, and the USDA, Soil, Plant Nutritional Laboratory, Ithaca, New York. The congenital deformity has been found to be caused by the ewes ingesting a poisonous range plant Veratrum californicum, on the 13th or 14th day after breeding. The ingestion of the plant before the 13th day of pregnancy does not seem to have any effect on the fetus, but the continued daily ingestion of the plant after the 14th day causes increased number of fetal deaths.

The cyclopian-type malformation in lambs has been occurring in southwestern and southeastern Idaho ever since the sheepmen have been breeding their ewes during the month of August on the mountain summer ranges. The loss has been estimated to range from $75,000 to $100,000 annually.

When the sheepmen were notified of the finding of this research work, they immediately changed their management practices to graze their ewes on ranges free from veratrum during the first 30 days of the breeding season. This practice completely eliminated their losses and the occurrence of the cyclopian-type deformity in their lambs, reduced the number of "dry ewes" due to fetal death and increased their lamb crop from 10 to 15 per cent.

The sheepmen cooperating on this project own from two to ten thousand ewes each and all reported this year to have had a lamb crop ranging from 160 to 165 per cent which has been the largest lamb crop in their sheep business career.

In the study to identify the substance in the veratrum responsible for the deformity, water, alcohol, and purified extracts were made from the dried ground stems and leaves, after which the extracts, residue and a recombination of all were individually fed to pregnant ewes daily from the 7th to 15th day after breeding.

All ewes fed the individual extracts and residue gave birth to normal lambs, but the feeding of the recombination of the extracts and residue caused cyclopian-type malformed lambs in 3 out of 9 ewes and fetal death in 2 ewes.

Studies are being continued with the National Animal Disease Laboratory and the Plant Nutritional Laboratory at Ithaca, to isolate and identify the causative agent.

Clinical observation suggests that the teratogenic agent may be a separate entity from the agent and/or agents responsible for the toxic symptoms in the ewe.

To date the congenital cyclopian-type deformity, as it occurs in lambs, has not been able to be reproduced in rats, rabbits or chick embryos. If a suitable laboratory animal could be found which would duplicate the results in the sheep it would serve as a valuable tool to enhance the study in isolating and identifying the causative agent.

The Veratrum californicum plants collected from 4 separate range areas at the same stage of growth showed a marked variation in their toxic effect on ewes and their ability to cause deformities in the lambs, as shown in the following table.

Summary of Feeding Veratrum californicum from four separate range areas to pregnant ewes from 7-15 days following breeding to determine toxicity of plant between range areas

Number of Animals	Source of Plant and Elevation	Grams Fed/Day	Toxic Symptoms In Ewes*	Fetal Development Number and Per Cent			
				Not Pregnant	Fetal Deaths	Deformed	Normal
14	Muldoon Canyon 62-6** 8200 ft.	140 gm.	Slight to Severe	4 or 28%	4 or 28%	4 or 28%	2 or 14%
20	Raft River Meadows 62-8 8000 ft.	140 gm.	Mild to Very Severe	4 or 20%	1 or 5%	1 or 5%	14 or 70%
5	Summit Flat 62-9 62-10 6100 ft.	140 gm.	Slight to Severe	0 or 0%	1 or 20%	1 or 20%	3 or 60%
8	Tony Grove 62-11 6300 ft.	140 gm.	Very Slight to Mild	1 or 12%	0 or 0%	4 or 50%	3 or 37%

*Classification of symptoms:
Slight = Grinding teeth and slight frothing.
Mild = Marked frothing and irregular gait.
Severe = Labored breathing, marked general weakness and/or prostrate.
**Collection numbers

2. Crooked Calf Syndrome. The crooked calf syndrome continues to be a serious disease problem throughout the range areas of the intermountain area. The etiological agent/or agents of this disease condition has not been determined. Based upon information accumulated to date and epidemiological studies appear that the lupine plant may be incriminated with this condition. Some ranchers feel that the crooked calf disease is hereditary in nature and transmitted by the bulls. Other ranchers have no concept or idea of the cause of this disease. This year one aborted moderately mascerated embryo from the experimental animals had a left front forelimb that was shorter and thicker than the right front forelimb. The malformed aborted embryo was from a cow that had been experimentally fed lupine and lead acetate from the 22nd to the 120th day of gestation. Eighty-seven malformed calves were born from cows that had grazed one range area in northeastern Utah. The clinical syndrome and pathology associated with the crooked calf disease appears to be somewhat characteristic in nature. The crooked calf disease is of great economic importance to the livestockmen of the intermountain area and also has invaluable implications of affording insight into skeletal deformities and cleft palates of animals and man. The crooked calf syndrome is estimated to cost the livestock industry from $100,000 to $200,000 annually.

3. Locosis. Range livestock losses from loco poisoning have long been a problem in the intermountain area. Serious losses from locosis, however, have been periodic in nature. The cause of this is unknown. In the past few years increase in the loco plant population and the losses due to Oxytropis sericea has caused much concern to livestock men in the intermountain area. Loco is a word of Spanish origin, meaning crazy. This word describes the animal so affected and is also used as the name of the plant causing the disease. However, there are many plants that are often included under the common name of loco. These plants belong to the genera Oxytropis and Astragalus. Definite characteristic clinical symptoms were produced in the animals experimentally fed Oxytropis sericea. Correlation was made between time, dosage, and clinical syndrome. Additional information is badly needed regarding the pathology and functional disturbances associated with locosis. There is no known prevention or treatment for locosis at this date. The plants are so widely spread that it is impractical to control the plant by the use of herbicides based on data known to date. Further research is necessary to determine more about this disease process so that sound, economical, and useful advice can be given to the ranchers in the area where this disease is a problem in helping alleviate locosis in their livestock.

4. Effect of Feeding a Variety of High and Low Saponin Alfalfa Hay to Holstein-Friesian Bull Calves. The experiment was carried out in cooperation with the Crops Research Division at Logan, Utah. The "DuPuits," high saponin content, and "Lahontan," low saponin content, varieties of alfalfa were each fed to a group of 4 Holstein-Friesian bull calves 3 to 4 months of age for 5 months. Neither variety caused any toxic or physiopathological

changes in the animals' bodies, indicating the high saponin "DuPuits" variety of alfalfa hay can be ingested by cattle without harmful effects. (ADP a7-7(Rev.)

I. Alleviators and Diagnostic Tests for Plant Poisoning.

An experiment on the residual level and the histopathological changes in the tissue of sheep fed 2-4D acid (Technical 2,4-Dichlorophenoxyacetic Acid, 99.0% Purity) for 30, 60, and 90 day periods, was carried out in cooperation with the Division's Station at Kerrville, Texas. At the time of this report only the results of the tissues from the animals fed 2 grams of 2-4D acid equivalent for the 30-day period was completed. Chemical analysis to determine the 2-4D residue in the body tissues were made at the Toxicological Laboratory at Kerrville. Kidney, rumen, renal fat, and body fat samples gave readings less than the same tissues from the untreated control. Muscle samples averaged less than 0.3 p.p.m, and livers less than 1.0 p.p.m. above the control. No histopathological changes were observed in any of the organs. (ADP a7-17)

J. The Susceptibility of Wild Animals to Foot-and-Mouth Disease

The virus content of an animal's blood may be used as one criterion for determining susceptibility to foot-and-mouth disease. If an animal is susceptible to the disease, the inoculated virus will multiply and produce a higher titer in blood than would be found if the virus only was being diluted by the animal's blood and tissues. Before such studies with wild animals can be evaluated, the results with susceptible or natural hosts should be determined. For this reason viremia studies were made with cattle. Virus was found in blood from as early as 4 hours to as long as 5 days after inoculation. For the first 2 hours after inoculation, vur was not detected. The peak of viremia usually was between 24 to 48 hours after inoculation. After 72 hours, the virus titer usually declined rapidly. This information will be used as a base line for viremia studies in wild animals. (ADP a7-21)

K. Mycotic Diseases of Domestic Animals.

Two species of Nocardia, Nocardia asteroides and Nocardia brasiliensis, have been reported frequently as the causes of local and systemic infections of man and animals. Cattle appear to be the most frequent animal host; nocardial mastitis is the predominant infection in cattle with bovine farcy and pulmonary nocardiosis next in order of frequency. Nocardiosis of dogs constitutes a major portion of animal infections; cutaneous, thoracic, and abdominal infections are most frequent.

N. asteroides and N. brasiliensis are both soil borne organisms; infection results from inhalation of Nocardia-laden dust or from direct introduction of the organism through flaws in the skin. N. brasiliensis infection is rarely recognized in animals.

Lesions of systemic nocardiosis are usually chronic processes characterized by suppuration and abscess formation. The degree of granulomatous response depends largely upon the host species, the organ infected and the duration of infection. Nocardial mastitis in cattle appears to be a clinical intermediary between systemic Nocardiosis and Nocardial mycetoma.

Recognition of nocardial infection is dependent upon proper isolation and identification. The interposition of Nocardia between the genera Mycobacteria and Streptomyces often complicates proper identification. Immunologic responses to nocardiosis have been studied in cattle. Delayed cutaneous hypersensitivity to a culture filtrate antigen and the use of this antigen in complement-fixation tests has facilitated the diagnosis of bovine nocardiosis.

In cooperation with Pathology Investigations of this Laboratory (NADL), an investigation of a severe skin disease of local dairy cattle was made. The condition was found to be cutaneous Streptothricosis caused by the organism Dermatophilus congolensis. The causative organism was isolated from active lesions, characterized and compared with isolates from cases recently described in New York. Transmission studies were conducted in experimental animals and histological characterizations were made of the natural and experimentally induced lesions. Attempts to isolate the organism from the environment of the affected herd were unsuccessful. (ADP a7-24)

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AREA NO. 8 - FOOT-AND-MOUTH AND OTHER EXOTIC INFECTIOUS DISEASES OF CATTLE

Problem. Responsibility for protection of the Nation's livestock industry against diseases, including those of foreign origin, was delegated to the USDA in 1884. Thereafter, contagious bovine pleuropneumonia eventually was eradicated from the United States, thus reopening European markets for American cattle. Ever since then the Department has successively imposed practicable, scientifically justified barriers against introduction of such dangerous exotic diseases as foot-and-mouth disease and rinderpest. The Plum Island Animal Disease Laboratory was established for scientific support of measures for protection against these and other foreign diseases of animals, following the direct threats of spread of foot-and-mouth disease from Mexico and Canada (1946-1954). Foot-and-mouth disease, which is capable of reducing overall productivity by 25 percent in areas where it becomes established, persists in most major livestock-producing countries, except Central and North America, Australia, and New Zealand. Rinderpest continues to be a serious disease problem in Africa and Asia; it is capable of killing 90 percent or more of the cattle that are exposed to it. Other diseases, such as contagious bovine pleuropneumonia, Rift Valley fever and East Coast fever continue to exact severe tolls in other parts of the world. Possibilities of entry of these diseases into the United States continue, despite all precautions, primarily because of the progressively increasing scope, speed and extent of modern international transportation. The purposes of the Plum Island laboratory are development of basic information applicable to protection of the Nation's livestock from foreign animal diseases; development and maintenance of competence in diagnosis of these diseases, and fundamental research on the biological, chemical and physical properties of the infectious agents that may be useful in prevention, control and eradication of these diseases.

USDA PROGRAM

The Department has a continuing long-term program involving veterinarians, biochemists, biophysicists, microbiologists, and pathologists, engaged in basic and applied research in this problem area. All of this research is being conducted on the following diseases at the Plum Island Animal Disease Laboratory, Greenport, Long Island, New York, except for supplemental field studies on vaccines in The Netherlands.

The Federal scientific effort devoted to research in this area, conducted solely at the Plum Island Animal Disease Laboratory, totals 25.0 professional man-years. This effort is divided among sub-headings as follows:

Pathology -- foot-and-mouth and other exotic diseases 1.0

Fluorescent antibody technique to locate viruses 1.0

Studies of foot-and-mouth disease vaccine 4.0

Immunological investigations to determine the mechanism of antibody formation using viruses of exotic animal diseases 0.5

Immune response to types and sub-types of foot-and-mouth disease virus 1.5

Quantity production of foot-and-mouth disease virus 2.0

Microcinematography of infected cells 0.5

Establishment and characterization of cell lines and cell strains 1.5

Interaction between foot-and-mouth disease virus and host cells 1.0

Genetic biochemistry of foot-and-mouth disease and other exotic viruses 1.0

Effects of natural and artificial stresses on foot-and-mouth disease virus 1.0

Bulk freeze-drying of foot-and-mouth disease virus vaccines and anti-serums 1.0

Rinderpest of cattle 2.5

Survival and transmission of foot-and-mouth disease virus in semen 1.5

Identification, purification, characterization of foot-and-mouth disease virus 2.0

Immuno-chemical investigations of foot-and-mouth disease 1.0

Survival and inactivation of foot-and-mouth disease virus in meat and meat by-products 1.0

Biological mechanisms of natural resistance and susceptibility to foot-and-mouth disease virus 1.0

Work was continued under a PL 480 grant to the Biological Institute, Sao Paulo, Brazil, for a 5-year study of tissue culture of indigenous strains of foot-and-mouth disease virus, and experimental field vaccination.

REPORT OF PROGRESS FOR USDA AND COOPERATIVE PROGRAMS

A. Pathology - foot-and-mouth and other exotic diseases.

The lingual pathology produced by the virus of foot-and-mouth disease was studied in 165 specimens using a technique designed to record and correlate gross, subgross, and microscopic characteristics of large numbers of lesions. The essential pathological alterations consisted of necrosis of epithelial cells in the stratum spinosum, intercellular edema and granulocytic infiltration. These changes caused development of circumscribed, slightly elevated blanched areas in the linqual mucosa, to which the designation "initial lesion" was applied. A considerable proportion of initial lesions developed promptly into full blown vesicles by separation of the diseased mucosa from the underlying tissue, and the fluid was probably lost through cracks in the stratum corneum. In such areas the desiccating, necrotic mucosa became discolored and the lesion had the gross appearance of a necrotizing rather than a vesicular process. This peculiarity of lesion development was attributed to firm attachment of the thick bovine lingual mucosa by the numerous, well-developed conical papillae.

Lesions in the interdigital skin were basically similar in their initial development but failure to vesiculate was exceptional. In this area, the necrotic edematous skin pulled away easily from the dermal papillae and large vesicles developed. The stress and motion to which the interdigital skin is subject was probably an important contributing factor. (ADP a8-1(R).

B. Fluorescent Antibody Technique to Locate Viruses.

An indirect fluorescent antibody (FA) technique for foot-and-mouth (FMD) evaluation of cattle serums was studied. The reacting system included commercial fluorescein-conjugated rabbit anti-bovine globulin, calf kidney cell cultures infected with foot-and-mouth disease virus (FMDV), rhodamine bovine albumin and test cattle serum. Serums from 55 cattle were evaluated for FA reaction and the results were related to FMD virus experiences of the animals and to their serum neutralization indices.

Serums from cattle that had developed lesions of FMD consistently gave positive or (in two instances) suspicious FA reactions. The different types of FMDV could not be distinguished one from another by the FA reaction when serums of cattle infected with the 7 types were used. The FA reaction was detectable in serums from two steers as early as 6 days and in the serum of one steer as late as 2 years after inoculation with FMDV. One serum tested two years after inoculation gave a negative FA reaction.

Serums from cattle that had not developed lesions of FMD consistently gave negative FA reactions. This included cattle in the following groups: normal controls, convalescing from vesicular stomatitis and virus diarrhea, and animals immunized with an experimental vaccine. (ADP a8-2(R)

C. Studies of Foot-and-Mouth Disease Vaccine.

The efficacy of swine, bovine, and baby hamster kidney cell cultures for the isolation, growth and assay of foot-and-mouth disease virus has been determined. These studies were performed with virus newly isolated from infected cattle. Swine kidney cells were preferable to bovine cells for virus growth and assay when low passage virus was used. Baby hamster kidney cells were very satisfactory and have the advantage that they can be produced in continuous culture. This cell line should prove to be a valuable asset in vaccine investigations.

Studies on the inactivation of foot-and-mouth disease virus indicate that formaldehyde does not reliably kill the virus. The more sensitive cattle tongue inoculation procedure for detecting possible residual live virus in formaldehyde treated preparations revealed that, while tissue culture and suckling mice tests gave no evidence of live virus, the cattle test readily demonstrated it. This more sensitive test thus indicated that formaldehyde was of questionable value for preparation of vaccines of assured safety. The compound, acetyl-ethylene-imine was used to prepare a lot of vaccine that did not infect cattle. This preparation is under potency evaluation. Other inactivating agents will also be examined to determine their possible use for producing vaccines.

Studies to establish the relative immunizing value of different vaccine preparations have been performed. Small animals, e.g., guinea pigs, are being used to circumvent the expense and difficulty of performing such tests in cattle. These studies revealed that the antibody produced by guinea pigs in response to the vaccine changed in its physical, chemical and serological characteristics with time following inoculation. As a result, interpretation of the results will be difficult until the significance of the two different types of antibodies produced is established.

Cooperative research on the extent of immunity of foot-and-mouth disease vaccine conducted in Amsterdam, Holland, revealed that knowledge of the extent and duration of immunity following vaccination against FMD is essential for the proper evaluation of vaccines and scheduling of field vaccinations. Because large numbers of animals are required over long periods of time, such studies can best be pursued in areas where the disease is enzootic and where field vaccination is routinely practiced. Studies in Holland in cooperation with The Netherlands Ministry of Agriculture have continued toward the evaluation of immunity of cattle vaccinated and held under field conditions. Twelve herds, consisting of approximately 400 cattle, are included in this study. Serum antibody levels against type O and A foot-and-mouth disease of animals vaccinated two or more times remain high over a 2-year period. Eighty per cent of those cattle which had received several annual field vaccinations and which were challenged 16-48 months later with virulent FMDV, showed resistance to the disease. In general there appears

to be a good correlation between serum antibody level and immunity, and studies will continue to further evaluate and define this relationship. (ADP a8-8(R)

D. Immune Response to Types and Sub-types of Foot-and-Mouth Disease Virus.

Cattle infected with foot-and-mouth disease continue to have virus neutralizing substances, i.e., antibodies, in their blood serum for at least five years after infection. Of three animals kept for this period following infection, one animal retained the ability to resist infection when re-exposed to the virus. Another group of cattle studied also demonstrated that antibody is present in sufficient amount for extended periods following infection. This was revealed by precipitation reactions performed by the agar gel method. It was also found that antibodies produced by cattle early in the course of an infection are different than those produced later.

It has been found that calves born of immunized dams do not have antibody to the virus in their serum; however, within 2 hours after receiving colostrum, antibody is found to be present. The transfer of antibody from dam to the calf's serum may be blocked by feeding skim milk or other proteins to the calf before it is allowed to receive colostrum from the vaccinated dam. It was not possible to immunize calves having moderate level of antibody circulating in their blood. It was necessary to let the colostrum-obtained antibody reach low level before satisfactory active immunization could be accomplished. This information is of importance in areas where foot-and-mouth disease occurs, as it is necessary to know at what age calves should be immunized for satisfactory protection. (ADP a8-11(R)

E. Quantity Production of Foot-and-Mouth Disease Virus.

A scheme for rapid modification of foot-and-mouth disease virus (FMDV) populations was developed by growth of the virus in progeny of tissue culture cells that survived infection. Plaque size diminished rapidly as virus was maintained in these cells, and, eventually, visible plaques were not produced. As modification of the virus population progressed, pathogenicity for mice and steers decreased with retention by the virus of significant levels of immunogenicity.

Some basic aspects of FMDV inactivation by glycidaldehyde (GDA) were investigated. The minimal concentration required to inactivate high concentrations of FMDV was between 0.008 and 0.12% GDA. The time required for inactivation of the virus was 105 minutes.

A major part of the effort devoted to this line project was spent in instructing and advising scientists in foreign countries on the growth of FMDV by tissue culture methods. Three months were spent in Turkey at the request of the United States Agency for International Development and the Government of Turkey. During this time a laboratory, consisting of 14 rooms was designed, established, and equipped with laboratory furniture, carts,

trucks, etc. More than 200 different laboratory items were ordered - mostly from the United States. Advice was given on organization of the laboratory and on production procedures.

One month was spent at the Razi Institute in Iran with personnel who requested advice on large-scale tissue culture media production.

Three weeks of instruction were provided at the Plum Island Animal Disease Laboratory to two scientists from Italy on production of FMDV by tissue culture methods. (ADP a8-12(R)

F. Microcinematography of Infected Cells.

Two black and white prints of each of the following films have been prepared:

Cytology I	-18676S	Cell Survival and Cell Culture Regeneration after Infection with Vesicular Stomatitis Virus - New Jersey Type.
Cytology II	-18675S	Cell Survival and Cell Culture Regeneration after Infection with FMDV-A119-BK8.
Cytology III	-18677S	Cell Survival and Cell Culture Regeneration after Infection with FMDV-A119-PB106.
Cytology IV	-18674S	Cytopathic Effect of Rinderpest Virus in Tissue Culture.

The following films (reversal film) have been prepared:

Cytology V	-18673S	Cytopathic Effect of Rinderpest Virus in Tissue Culture.
Cytology VI	-18672S	Cytopathic Effect of FMDV in Lamb Testicular Cells.

Titles will be redone and prints made of the last two films. (ADP a8-13(R)

G. Establishment and Characterization of Cell Lines and Cell Strains.

A lamb testis cell line developed at the Plum Island Animal Disease Laboratory, was used in microcinematographic study of cellular reactions to infection with foot-and-mouth disease virus (FMDV). This lamb testis cell line was also used in the development of fluorescent antibody technique to locate FMDV in the cell. (ADP a8-14(R)

H. Interaction Between Foot-and-Mouth Disease Virus and Host Cells.

Analytical ultracentrifugation was used to assess the efficacy of physical and chemical separations of 7S and 19S antibodies in sera of guinea pigs

and cattle convalescing from foot-and-mouth disease. A one-step preparative ultracentrifugal procedure separated the two antibody classes. The precipitin reaction was used to determine antibody stability in several solvents.

Foot-and-mouth disease virus was labeled with $P^{32}O_4^{=}$ and leucine-3,4-H^3. Three classes of RNA with sedimentation coefficients ($s_{20,w}$) of about 4S, 12S and 23S have been isolated from bovine-kidney culture cells.

A stable baby hamster kidney cell line, obtained from the University of Glasgow, was grown in inexpensive media in roller bottles to populations of about 625 million cells per bottle in 6 days without fluid change. Infection with FMDV yielded fluids containing $10^{8.5}$ to $10^{8.8}$ PFU/ml. It appears feasible to scale the method upward to produce many milligrams of virus during a single week.

Suckling mice which survive one inoculation with high dilutions of FMDV which still contain appreciable numbers of physical virus particles die when reinoculated 3 to 4 days later with the same statistics as control animals. This suggests that not all FMDV physical particles are lethal and that only those mice die which receive a lethal particle amongst the many administered. (ADP a8-17(R)

I. Genetic Biochemistry of Foot-and-Mouth Disease and Other Exotic Viruses.

Heat denaturation of RNA obtained from virtually pure FMDV by phenol treatment was indicative of pure single-stranded RNA. Its temperature of half-melting (T_m) in 0.02 M and 0.05 M sodium phosphate at pH 7.5 was 55° and 59°C, respectively. Such RNA contained guanine, adenine, cytosine and uracil in the molar fractions 0.24, 0.26, 0.28 and 0.22. RNA within FMDV did not heat denature until after its release from the protein coat. In 0.05 M sodium phosphate at pH 7.5 this commenced abruptly at 54°C. The T_m was 70°C. Redenatured virus melted very similarly to phenol-derived RNA with a T_m of 59°C.

Guanidine reversibly decreased FMDV production in bovine kidney cultures. This inhibition occurred during the latter stages of virus maturation and was not reversed by arginine or urea, both of which are structurally related to guanidine. Virus adsorption by cells was not affected. Para fluorophenylalanine inhibited virus reduplication in cells grown in serum-free medium, but not when serum was present. 2,6-diamino-3 phenyl-azopyridine hydrochloride (Pyridacil) reduced virus production possibly through its cellular toxicity. (ADP a8-18(R)

J. Effects of Natural and Artificial Stresses on Foot-and-Mouth Disease Virus.

Trichlorofluoroethane (TTE) and chloroform are each capable of reactivating virus from a neutral mixture of virus and specific antiserum. One part of

the chemical TTE was mixed with two parts of the virus-serum mixture. The material was then centrifuged and the aqueous layer recovered. Such treatment after eight extractions yielded the maximum virus in the aqueous layer. Butanol was found to inhibit the action of TTE or chloroform to extract the virus from a neutral mixture.

Two subcultures of type A, strain 119 FMDV adapted to tissue culture were compared for stability. After 90 passages, the two lines differed when exposed to 60 C; one was inactivated in 60 minutes and the other in 30 minutes. The two subcultures differed in response to drying on slides at 37C and 20% relative humidity; one survived over 120 days while the other was inactivated in 35 days. Both sublines of virus reacted alike to ultraviolet irradiation and were neutralized by the same antiserum.

Under the conditions of testing, the presence of phenol red in the virus medium did not modify the inactivation time of the virus by ultra-violet light. However, centrifugation at 10,000 r.p.m. for 15 minutes and subsequent exposure of the supernatant to ultraviolet light showed the virus in such preparations to be inactive after 2-hours treatment. Specimens not centrifuged had three logs of virus remaining after 3 hours exposure to ultraviolet light. Twenty ml volumes of virus suspension in a petri dish were used and the fluid film was 0.5 cm thick at a distance of 8 inches below the germicidal light. The intensity was 7/u watts/sq.cm. Tongue tissue suspensions of virus were centrifuged and exposed to UV under the same conditions as the tissue culture virus with the result that 2.5 logs of viral activity remained after 105 minutes of exposure to UV. (ADP a8-19(R)

K. Bulk Freeze-Drying of Foot-and-Mouth Disease Virus, Vaccines, and Antiserums.

Type A, strain 119 foot-and-mouth disease virus, dried in tissue culture fluid in 250 ml amounts at either chamber temperature or with a 37C heat input to the drying chamber, did not process or store well at 4, 23, or 37C for 3, 2 or 1 months, respectively. This is in contrast to the same virus in 4 ml volumes in ampules which showed no loss in titer after 29 months of storage at 4C. (ADP a8-20(R)

L. Survival and Transmission of Foot-and-Mouth Disease Virus in Semen.

Sixteen grade Hereford bulls were infected with foot-and-mouth disease virus (FMDV) by tongue inoculation. The virus strains used represented six of seven known types of FMDV. At various times after inoculation, semen was obtained from bulls by electroejaculation for inoculation into steers and suckling mice to recover virus and determine titers, and for insemination of heifers.

Foot-and-mouth disease virus was found in semen of 2 bulls as early as 12 hours postinoculation which was prior to appearance of clinical signs of infection. Thereafter, virus was found in semen of the 16 bulls in 55 of

65 attempts, for as long as 10 days postinoculation. The virus titer in semen was usually higher than in urine and sometimes higher than in blood samples taken simultaneously.

Artificial insemination techniques were used to place semen from infected bulls in cervical canal and vagina of 19 heifers. Four of the 19 heifers developed FMD. It was concluded that semen of bulls could contain FMDV prior to appearance of clinical signs and lesions of infection and that FMD may be transmitted by artificial insemination. (ADP a8-24)

M. Identification, Purification, Characterization of Foot-and-Mouth Disease Virus.

Foot-and-mouth disease virus was produced in roller bottle bovine kidney cultures. About 3 liters of virus were harvested each week containing $10^{8.8}$ plaque-forming units per ml. Chemical and ultracentrifugal concentration and purification yielded 2 mg virus of at least 94% purity with maximal infectivities of $10^{11.0}$ PUF/ml., specific infectivities of $10^{14.0}$ PFU/gm and physical particles by electron microscope counting of $10^{13.6}$ virus particles/ml. The virus contained 32% ribonucleic acid (RNA) and 68% protein and had a 1% extinction coefficient at 259 mu of about 76. Its specific refractive increment was about 0.16 ml/gm. Viral RNA had an extinction coefficient at 258 mu of approximately 220 and a max_{258}/min_{230} ratio of 2.1. The protein coat subunit of the virus obtained by heat, acid or urea treatments had a sedimentation coefficient ($s_{20,w}$) of 12.2± 0.3S. There is considerable, but yet unequivocal electron microscope evidence that FMDV is a icosahedron with 42 subunits in its coat protein. (ADP a8-25)

N. Immuno-Chemical Investigations of Foot-and-Mouth Disease.

Antibodies present in the blood serum of animals immunized against, or infected with, foot-and-mouth disease virus are known to play a major role in their resistance to subsequent exposure to the virus. It has been shown that these antibodies may be of two different types, and they appear at different times following initial exposure to either the living or inactive form of the virus. These two types of antibodies have different physical and chemical characteristics. The early appearing antibodies are larger molecules (19S sedimentation rate) than the later developed ones (7S sedimentation rate). They also migrate more rapidly under the influence of an electrical field (B-globulin mobility) than do the later ones (y-globulin mobility). Other differences in physical-chemical characteristics have also been found. In addition, differences in their serological activity have been found. The late appearing antibody is able to fix complement while the early antibody is not. The latter finding is important in that it imposes a limitation upon our diagnostic capabilities. Knowledge of the physical, chemical, and immunological nature of these antibodies is vital for an understanding of how animals resist infections.

Progress on the immunological characterization of the foot-and-mouth disease virus and antibodies produced by animals in response to the virus is dependent upon the development of precise measuring techniques. Quantitative immunological measuring methods have been applied to purified and crude virus preparations successfully. These procedures have certain advantages over many regular physical and chemical methods in that only minute amounts are required for the tests, the tests are usually easier to perform, and they may often be done on samples containing contaminating substances that interfere with other assay procedures. Further refinement and application of these procedures will increase our knowledge of the structure of foot-and-mouth disease virus. (ADP a8-26)

O. Survival and Inactivation of Foot-and-Mouth Disease Virus in Meat and Meat By-Products.

It has been established that the primary sites where foot-and-mouth disease virus may survive in carcass and in boned meat from infected animals are lymph nodes, hemal nodes, blood clots and bone marrow or bone fragments. A new and previously unreported site for virus survival is the joint fluids. Virus was found in joint fluids as early as 12 hours and for as long as 5 days after inoculation of cattle. The virus survived in joint fluids throughout usual treatments given carcass meat. During boning operation, virus in joint fluids could contaminate butcher's knives and be spread over surfaces of cut meat. The protection afforded the virus by the joint fluid would permit virus survival under some adverse conditions on surface of meat chunks. Thus, virus in joint fluids could be a hazard in imported meat. (ADP a8-28)

P. Biological Mechanisms of Natural Resistance and Susceptibility to Foot-and-Mouth Disease Virus.

Mice vary in susceptibility to infection with foot-and-mouth disease virus. Factors which affect the susceptibility or which might be related to the variation in response have been investigated. (1) Suspensions of kidney cells from 1- and 2-week old mice produced FMDV earlier and to higher titers than cells from older mice. While 1- and 2-week old mice are in the most susceptible age range, this difference in virus multiplication might be related to factors other than the susceptibility of cell donors. (2) Mother mice are most susceptible to FMDV during the first two weeks post partum and then gradually become resistant. To determine if this development of resistance was associated with weaning of their young and a consequent decrease in milk production, a group of mothers was given new litters of 5- to 7-day old mice at weekly intervals to the 4th week post partum. Challenge with FMDV demonstrated that such mice were slightly more susceptible than similar mothers with original litters but much less susceptible than 6-day post partum mothers. (3) Pregnant as well as mother mice are susceptible to FMDV. Experiments demonstrated that mother mice maintained the high degree of sensitivity to bovine serum which they developed before mating but became less sensitive after delivery of their young. (ADP a8-29)

Q. Studies on Foot-and-Mouth Disease Virus.

A PL 480 Grant was made to Instituto Biologico, Sao Paulo, Brazil, to conduct studies on foot-and-mouth disease virus. A laboratory has been established for production of tissue cultures to use as a media for propagating foot-and-mouth disease virus, and in the routine isolation of the virus from specimens received from the field.

A swine kidney cell line has been propagated for more than 18 months. The cells have remained fully sensitive to at least 3 types of FMDV encountered in Brazil. Work on attenuation of FMDV by tissue culture methods in this laboratory is continuing but no significant findings have thus far been reported. (S3-ADP-2)

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AREA NO. 9 FOOT-AND-MOUTH AND OTHER EXOTIC DISEASES OF SWINE

Problem. Foreign diseases, such as foot-and-mouth disease, African swine fever, and Teschen disease, that occur elsewhere in the world, constitute calculable potential threats to the swine industry of the United States. Foot-and-mouth disease is of particular importance because the disease frequently occurs primarily in swine from which it spreads to other susceptible species, such as cattle and other ruminants. African swine fever, which until recently was confined to wild and domestic pigs in Africa, has spread to Portugal and Spain. The disease is of special concern because of its resemblance to hog cholera, with which it may be confused. Moreover, mortality from the disease approaches 100 per cent, and there is no specific preventive vaccine. Teschen disease, which causes widespread inapparent infections and occasional involvement of the central nervous system, is another of the foreign diseases to be guarded against. A disease indistinguishable from Teschen disease has appeared in England in recent years. Despite all precautions, any of these diseases may occur in the United States, as likely as not through the medium of modern, rapid international transportation. The Plum Island Animal Disease Laboratory is engaged in studies of foreign diseases of swine, for the purpose of developing information for increased protection of the Nation's swine industry.

USDA PROGRAM

The Department has a continuing long-term program involving veterinarians, biochemists, microbiologists, and pathologists, engaged in basic and applied research in this problem area. Research is being conducted on the following diseases at the designated locations.

The Federal scientific effort devoted to research in this area totals 6.6 professional man years. This effort is divided among sub-headings as follows:

Foot-and-Mouth Disease of Swine 1.0 at the Plum Island Animal Disease Laboratory, Plum Island, New York.

African Swine Fever 4.6 at the Plum Island Animal Disease Laboratory in cooperation with the East African Veterinary Research Organization, Muguga, Kenya, and in connection with a PL 480 project in Madrid, Spain, where the equivalent of $97,550 has been made available to the Spanish Ministry of Agriculture over a 3-year period.

Rinderpest in pigs 1.0 at the Plum Island Animal Disease Laboratory, Plum Island, New York.

REPORT OF PROGRESS FOR USDA

Foot-and-mouth Disease of Swine

No report for this reporting period. (ADP a9-1(Rev.)

A. African Swine Fever

As the result of work with killed and attenuated vaccine, anti-African swine fever serum to 5 isolates of the virus is now available. Six isolates of the virus have been adapted to pig kidney culture and 3 have been attenuated by serial passage in leucocytes to produce survivor animals for serum production and cross-challenge studies. Of the 35 isolates with African swine fever virus presently available, 11 originated in domestic pigs, 17 in wart hogs, 5 in bush pigs and 1 each in porcupine and hippopotamus. The hyena may also be implicated as a carrier of African swine fever.

African swine fever infected tissue culture fluid may be fractionated to separate the virus with an 80-fold increase in purity and yield 3 non-infectious fractions. One of the fractions reacts in the agar gel test with 4 different antisera; another is specific only with early convalescent serum, and the third specific only with homologous sera. Each fraction has been inoculated into rabbits to produce antisera.

In comparative studies of African swine fever and hog cholera, it has been shown that the two diseases are strikingly similar. It has also been shown that pigs immune to African swine fever virus are fully susceptible to hog cholera virus. This method may be used to differentiate the two.

In thermal studies with the virus it has been shown that African swine fever virus remained infective after treatment at 56°C for 80 minutes but not after 120 minutes. The virus was not infective after heating at 81°C for 2 minutes. (ADP a9-2(Rev.)

During the life of the PL 480 agreement, the Spanish have examined the test rather exhaustively in that 803 samples have been tested. Of these 803, 595 samples were positive to the hemadsorption test. Sixty-eight percent of the positive samples were examined and African swine fever virus was demonstrated in each sample. Confirmation was obtained by inoculating swine with the test material. Similarly, 49 samples which were negative to the hemadsorption test, were examined by animal inoculation and all 49 samples were shown to be free of African swine fever virus. (E25-ADP-4)

B. Rinderpest in Pigs

Little attention has been given to the study of rinderpest in pigs. This indifference probably stemmed from the unfounded belief that European breeds of swine were not susceptible and because management practices in rinderpest

infected areas populated with European pigs do not lend themselves to extensive mixing of cattle and swine.

Results of work at the Plum Island Animal Disease Laboratory show that pigs may acquire "silent" infection from cattle, carry rinderpest virus for approximately 2 weeks, and thereby provide a dangerous source of infection. Continued passage of the virus in pigs does not appear to increase its virulence.

Studies with blood of rinderpest-infected pigs have resulted in findings which may be used for diagnosis. Post-mortem examination of pigs infected with rinderpest virus are of little diagnostic value because tissue changes are minimal. This observation led to the conduct of a study to determine if microscopic tissue changes could be detected. Those organs with lymphoid tissues were found to be affected and the character of the change is sufficiently distinctive for use in differential diagnosis.

Pigs did not show clinical signs of infection when inoculated with a tissue culture modified strain of rinderpest virus which was developed for use as an immunizing virus for cattle. (ADP a9-3)

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AREA NO. 10 - FOOT-AND-MOUTH AND OTHER EXOTIC DISEASES OF SHEEP

Problem. For the early detection of any outbreak of foot-and-mouth disease, comprehensive information regarding its effect on all susceptible species is necessary. The effect of foot-and-mouth disease (FMD) on cattle and swine has been, and is being investigated, however, little information is available pertaining to the disease in sheep. Sheep infected with FMD could serve as a source of infection and initiate the spread of the disease. Although primary research emphasis on exotic diseases of sheep at the Plum Island Animal Disease Laboratory is on FMD because of its great economic importance, other exotic diseases of sheep, such as rinderpest, sheep pox, louping ill, Nairobi sheep disease, and Rift Valley fever, are of concern to the Plum Island Laboratory because techniques and materials may be needed for diagnosis, control, and eradication on short notice and unexpectedly. Such diseases, if introduced into this country, could result in high death tolls or cause serious economic losses among susceptible sheep and other livestock. The problem is one of development of basic information applicable to protection of the nation's sheep from foreing animal diseases; development and maintenance of competence in diagnosis of these diseases, and fundamental research on the biological, chemical, and physical properties of the infectious agents that may be useful in prevention, control, and eradication of these diseases.

USDA PROGRAM

The Department has recently activated a continuing and long-term program involving veterinarians, biochemists, microbiologists, and pathologists, engaged in basic and applied research in some of the problems in this area.

The Federal scientific effort devoted to research in this area totals 2 professional man years. This effort is divided among sub-headings as follows:

Foot-and-Mouth Disease of Sheep,1.0 at the Plum Island Animal Disease Laboratory, Greenport, Long Island, New York.

Rinderpest in Sheep, 1.0 at the Plum Island Animal Disease Laboratory, Greenport, Long Island, New York.

REPORT OF PROGRESS FOR USDA AND COOPERATIVE PROGRAMS

A. Foot-and-Mouth Disease in Sheep

At the Plum Island Animal Disease Laboratory, the clinical and serological responses of sheep after infection with foot-and-mouth disease virus (FMDV) are being determined. Sheep were shown to be susceptible to infection with FMDV by inoculation and by contact exposure. The disease in sheep was characterized by fever and the development of vesicular lesions involving the oral mucosa and coronary borders of the feet. Infected near-term ewes did

not abort. Hematological changes were those associated with infections characterized by neutrophilia; there was no evidence of leukopenia.

Virus-neutralizing, complement-fixing, and precipitating antibodies were detected in the serums of sheep following infection with FMDV. Investigations have been and are being conducted on the persistence of these antibodies. Virus-neutralizing antibodies were still present 462 days post-inoculation; precipitating antibodies 378 days post-inoculation. The persistence of antibodies in sheep following infection with FMDV and the fact that these antibodies may be readily detected is significant from a regulatory standpoint. A lamb testis cell line developed at PIADL was used in micro-cinematographic study of cellular reactions to infection with FMDV. This lamb testis cell line was also used in the development of fluorescent antibody technique to locate FMDV in the cell.

A film was prepared at PIADL on the Cytopathic Effect of FMDV in Lamb Testicular Cells.

B. Rinderpest in Sheep

At the Plum Island Laboratory work has been instigated to determine the effects of a bovine strain of rinderpest virus on sheep raised in the United States. Sheep are not highly susceptible to rinderpest virus, hence detection of exposed animals is difficult. Studies with experimentally infected sheep have shown that rinderpest virus may be recovered from the blood for 10 days after inoculation. Tests in cattle with blood taken during this period would be of value in diagnosis.

Sheep did not show clinical signs of infection when inoculated with a tissue culture modified strain of rinderpest virus which was developed for use as an immunizing virus for cattle. The modified virus prompted production of rinderpest antibodies in sheep.

PUBLICATIONS REPORTING RESULTS OF USDA RESEARCH

Hess, W. R., H. J. May, and R. E. Patty. 1963. Serial Cultures of Lamb Testis Cells and Their Use in Virus Studies. Amer. J. Vet. Res., 24: Jan., 59-64.

AREA NO. 11 - PARASITES AND PARASITIC DISEASES OF CATTLE

Problem. The cost of parasitic diseases to the cattle industry of the United States is estimated to be in excess of $400 million annually. Disorders caused by parasites are ubiquitous, generally insidious and often overlooked entirely. Diagnosis is difficult and successful treatments for many of these diseases are not available. Moreover, management practices to avoid spread of parasitisms and to control them are often ineffectual. The problem is to develop, through a planned, balanced program of basic and applied research, knowledge for preventing, controlling or eradicating parasitic diseases so as to provide for healthy cattle, insure adequate supplies of parasite-free beef for an expanding population, avoid or minimize economic losses caused by these diseases, and thereby contribute to a more prosperous agriculture and the national economy.

USDA PROGRAM

The Department has a continuous long-term program involving biochemists, microbiologists, parasitologists, pathologists and veterinarians engaged in both basic and applied studies directed to the development of measures for the solution to the high and extremely costly incidence of parasitism in cattle. Research is being conducted on parasitic diseases at the designated locations.

The Federal scientific effort devoted to research in this area totals 21.5 professional man-years. This effort is divided among subheadings as follows:

Ecological Factors Influencing Nematode Development 1.0 at the Animal Disease and Parasite Research Division, Regional Animal Disease Laboratory, Auburn, Alabama, and through informal cooperation with the Georgia Experiment Station, Experiment, Georgia.

Effects of Pasture Mixtures and Pasture Management on Control of Internal Parasites 1.5 at the Regional Animal Disease Laboratory, Auburn, Alabama, and through informal cooperation with the Georgia Experiment Station, Experiment, Georgia.

Acquisition and Effects of Roundworm Parasites of Cattle, as Influenced by Diet 1.5 at the Animal Disease and Parasite Research Division, Beltsville Parasitological Laboratory, Beltsville, Maryland.

Artificial Propagation of Protozoan Parasites 1.0 at the Beltsville Parasitological Laboratory, Beltsville, Maryland.

Host-Parasite Relationships of Coccidia 1.0 at the Regional Animal Disease Laboratory, Auburn, Alabama.

Ecology and Immunology of the Cattle Lungworm 1.0 at the Beltsville Parasitological Laboratory, Beltsville, Maryland.

Clinical and Physiological Aspects of Roundworm Parasitism in Cattle 2.0 at the University of California, Davis, under a cooperative agreement with the USDA.

Investigations of Trichomonad Parasites 1.0 at the Animal Disease and Parasite Research Division, Regional Animal Disease Laboratory, Logan, Utah, and under a cooperative agreement with the Utah Agricultural Experiment Station, Logan.

Host-Parasite Relationship of Intestinal Worms Cooperia spp. 2.0 at the Regional Animal Disease Laboratory, Auburn, Alabama.

Factors Influencing Internal Parasitism of Grazing Cattle 1.5 at the Beltsville Parasitological Laboratory, Beltsville, Maryland.

Winter Coccidiosis (Bloody Scours) 1.0 at the Regional Animal Disease Laboratory, Logan, Utah, and under a cooperative agreement with the Montana Agricultural Experiment Station, Bozeman.

Anaplasmosis of Cattle 4.0 at the Beltsville Parasitological Laboratory, Beltsville, Maryland, and through a memorandum of understanding and other agreements in cooperation with State Experiment Stations in California, Illinois, Louisiana, Nevada, the State Veterinarian of Tennessee, the USDA Entomology Research Station, Kerrville, Texas, and The Delta Branch Experiment Station, Stoneville, Mississippi.

Investigations on Anaplasmosis, Piroplasmosis and Babesiellosis of Cattle, are under way through a PL 480 Grant, at the School of Veterinary Faculty, Montevideo, Uruguay.

The Interrelationship of Diet and Parasitic Infection in the Production of Cattle 1.0 at the Regional Animal Disease Laboratory, Auburn, Alabama.

The Histochemistry of Gastro-Intestinal Nematodes of Cattle 1.0 at the Regional Animal Disease Laboratory, Auburn, Alabama.

Parasites of Cattle - Stephanofilarial Species 1.0 at the Animal Disease and Parasite Research Division, Regional Animal Disease Laboratory, University Park, New Mexico.

REPORT OF PROGRESS FOR USDA AND COOPERATIVE PROGRAMS

A. Ecological Factors Influencing Gastro-intestinal Nematodes.

Investigations made at Experiment, Georgia, under the auspices of the Animal Disease and Parasite Research Division's (ADP) Regional Animal Disease Laboratory at Auburn, Alabama, showed a significantly greater number of larvae of T. axei was recovered from cultures made with feces when the host calf was on a hay diet than when it ate a grain (corn) ration. Grain added to feces containing eggs of T. axei and T. colubriformis completely prevented the development of larvae in cultures when compared to cultures without the grain additive. Undigested grain (corn) washed from the feces passed by a calf on a grain ration also inhibited the larval development when added to feces from a calf infected with T. colubriformis. Experiments have shown that a grain ration (corn) appears to have an inhibitory action on the development in the feces of the larvae of two species of cattle nematodes (Trichostrongylus axei, T. colubriformis). (Georgia)

Experiments at the ADP Regional Animal Disease Laboratory, Auburn, Alabama, have demonstrated that rabbits are infected by ingesting the third-stage larvae of the nematodes, Trichostrongylus calcaratus, T. affinis and Obeliscoides cuniculi. Rabbits do not become infected by placing larvae on the intact skin if precautions are taken to prevent ingestion of such larvae by the host. Trichostrongylus affinis has been found in the cottontail (Sylvilagus floridanus) at Auburn, Alabama. Preliminary studies indicate that this nematode inhabits the cecum and appendix and has a prepatent period of 10-11 days with maximum egg production between 11 and 14 days post-infection. In guinea pigs immunized to the ruminant parasite, Trichostrongylus colubriformis, the immune response apparently was directed against the parasitic third- and fourth-stage worms. On the other hand, parasitic third-stage larvae failed to stimulate any immunity in guinea pigs, fourth-stage larvae elicited a measurable degree of immunity, and fourth- and fifth-stage worms, combined, stimulated an almost total immunity. (Auburn, Alabama) (ADP b1-6)

B. Artificial Propagation of Protozoan Parasites.

Work was continued at the ADP Beltsville Parasitological Laboratory (BPL) on the artificial propagation of protozoan parasites for the purpose of determining essential metabolites. Studies to develop methods of producing, under artificial conditions, the blackheat parasite in large numbers have been hampered by the unavoidable appearance of yeasts in the cultures. Amphotericin-B, an antifungal antibiotic, was found to control yeasts in culture without damage to the parasites, provided cream from cow's milk was incorporated in the culture medium. Without cream, the parasites grew poorly, if at all. Cholesterol compounds which the blackhead parasite can use for food were without effect when used in lieu of cream. (ADP b1-22)

C. Ecology and Immunology of the Cattle Lungworm.

It was found at the Beltsville Parasitological Laboratory that double vaccination with irradiated larvae of the cattle lungworm can be effective in protecting calves from becoming appreciably infected with adults of this parasite subsequent to a massive exposure to vigorous infective larvae of the worm, such as occur on pastures. However, the procedure was not invariably effective under experimental conditions. Variability in its efficacy was not dependent on rate of irradiation of the vaccine within the limits that have been tested. Inherent individual variation among calves in capacity to develop resistance appears to be a significant determinant of the efficacy of vaccination.

Calves can become highly resistant or immune to the development of a mature cattle lungworm infection as a result of vaccination with infective larvae of a lungworm of sheep. The experiments on which this conclusion is based appear to be the first to show that one species of livestock can be immunized against one of its worm parasites by exposing it to infection with a worm that inhabits a different species. (ADP b1-24)

D. Clinical and Physiological Aspects of Roundworms in Cattle.

The School of Veterinary Medicine, University of California, Davis, under a cooperative agreement with the USDA, reported research to show: Phenothiazine prepared with an anhydrous aluminum chloride catalyst was found to be superior to phenothiazine N.F. and equal to purified phenothiazine when evaluated in mice infected with Nematospiroides dubius: The addition of the other insoluble impurities of phenothiazine N.F. to purified phenothiazine did not reduce the anthelmintic action of the latter product, and that thiabendazole was found to be highly efficient as an anthelmintic in clinical cases of gastrointestinal parasitism of cattle when used at a level of 100 mg/kg body weight.

Work was also conducted on iron metabolism and hemopoiesis. The studies, utilizing Fe^{-59} were conducted in order to evaluate the red blood cell survival times in lambs as determined by the method of Baker and Douglas and that of Pollycove and Mortimer. It was found that the latter method gives a longer mean survival (131 days) than the former method (81.0 days). Definitive studies in 2 lambs, using the method of Eadie and Brown, indicated mean red cell survivals of 141 and 143 days.

Body surface monitoring of spleen, liver, and bone marrow indicated that the spleen of sheep may act as a labile storage pool for iron utilized in hemoglobin synthesis.

Analysis of tissues for Fe^{-59} between 1.1 hr. and 7 days following intravenous injection of Fe^{-59} labeled plasma in lambs, revealed that 4 to 21% of the injected isotope was in the liver, 0.00 to 2.5% in the spleen, 0.00 to 16.5% in muscle, 0.10 to 2.1% in the kidneys, and the remainder was in plasma, bone marrow, and red cells. (California) (ADP b1-25)

E. Investigations of Trichomonad Parasites.

Research on this project at the ADP Regional Animal Disease Laboratory, Logan, Utah, resulted in the following findings: Electrophoretic methods used for protein separation were not practical for immunoelectrophoresis because large amounts of antigen are required for the immunodiffusion phase of the procedure. The apparatus constructed for this method was therefore adapted to a micro-method. The supporting medium was changed from starch gel to ionagar which does not allow separation of as many fractions or as sharp delineation as starch, but the separation is sufficient for immunodiffusion. For general antigenic characterization and comparison of antigenic makeup of various strains of T. foetus, the micro-method should suffice. By this method rabbit anti-T. foetus serum was separated into the 5 fractions. The diffusion phase of the technique has not been combined with the electrophoretic phase, but has been done separately and produces precipitin lines. For positive identification of antigens that may characterize or differentiate strains, the starch gel method may be more effective.

Anti-serum against T. foetus may be produced in rabbits by intravenous injections of washed live organisms. However, some rabbits do not tolerate the infection. Intracardial injections did not prove satisfactory, neither did the intraperitoneal method with any of the 4 strains of T. foetus used. The effort to produce antiserum against T. foetus in calves gave negative results.

Antigens for use with the gel diffusion phase of the immunoelectrophoretic technique to date have been the complete type consisting essentially of concentrated ruptured organisms. Three methods were used for rupturing the organisms--1) lysis by hypotonic solutions, 2) alternate freezing and thawing, and 3) blendor grinding. Acceptible antigens have been prepared from freeze-thawing. However, blendor-ground organisms gave most consistent results.

Gel diffusion reactions were produced in ionagar gel of several strengths prepared with several buffers of varying pHs. Therefore, it is anticipated that the gel diffusion procedure may be combined with the microelectrophoretic technique. In trials to date, however, considerable cross reaction between the two strains used have occurred. T. foetus was isolated from 10 of 280 samples taken from bulls during the year. This included herds under observation following known infection. (Logan, Utah)

Research was continued at the Utah Agricultural Experiment Station, Logan, under a cooperative agreement with the USDA, to learn more about pentatrichomonads that were isolated from the rumen and cecum of calves in northern Utah in 1961. This trichomonad grew in Diamond's modified Plastridge's, and cecal-extract media. No difference in response to different media or in morphology was found in the trichomonads from the rumen

and cecum. There were 3, 4, or 5 unequal anterior flagella, one of which was independent, a relatively high, full-length undulating membrane, a prominent costa, a narrow axostyle with long protruding tip and no chromatic ring, a subspherical or round-oval nucleus, and an oval parabasal body with one or more central granules. Mean measurements of 100 Bouin's-fixed, protargol-stained, rumen specimens from 24-hour Diamond's cultures were as follows: length, 7.8 microns (range, 5.7-10.0); width, 6.1 (4.2-7.9); protruding tip of axostyle, 4.0; anterior flagella, 9.7, 8.8, 7.2, 6.0, and 4.2; trailing flagellum, 5.4; nucleus, 2.5 by 2.0; parabasal body, 1.5 by 0.9; height of undulating membrane, 1.7. This trichomonad is similar to Pentatrichomonas hominis and may be identical with this species. (Utah) (ADP b1-26)

F. Host-Parasite Relationship of Intestinal Worms Cooperia spp.

Reported research from the ADP Regional Animal Laboratory, Auburn, Alabama, showed that calves inoculated with 250,000 Cooperia pectinata infective larvae made an average weight gain of only 6 pounds in 6 weeks, while non-inoculated controls averaged a gain of 25.3 pounds. Clinical signs of parasitism -- anorexia and enteritis as indicated by passage of abnormally soft stools -- appeared during the third and fourth weeks of infection, and these were accompanied by decreased levels of serum proteins and blood sugars. The effects were not as severe as those produced in previous studies wherein calves were inoculated with 350,000 infective larvae. Calves inoculated with 350,000 Cooperia oncophora larvae developed a mild parasitism, characterized by a short period of enteritis and some retardation on rate of gain. However, this parasite is not as pathogenic as a closely related species, C. pectinata. The intestinal worm, Cooperia pectinata, develops in the intervillar spaces of the duodenal mucosa, causing disruption of the intestinal villi, and evoking a catarrhal exudate.

It has been concluded from experiments at Auburn that some factor, or factors, operating within individual host animals (calves and lambs) are sufficient to affect significantly the size of infective third-stage larvae (Cooperia oncophora) developed from eggs laid by the infecting parasites. Some of these factors may be individual differences in the utilization of the diet by the host, or differences in the microbiota of the feces. (Auburn, Alabama) (ADP b1-27)

G. Factors Influencing Internal Parasitism of Grazing Cattle.

The Beltsville Parasitological Laboratory (BPL) research workers reported that calves and older cattle infected with nematodes were maintained on pasture in the summer and fall. During the first two months following infection, the rotated animals gained better than the non-rotated animals. This advantage was not maintained by the calves for the remaining two months of the experiments. In general, the worm burden of the rotated animals was as great as that of the non-rotated animals. The age of the animals had a greater effect on worm burden than rotation.

Mature cattle up to the age of at least 3 to 4 years are as susceptible to initial infection with the beef measles worm as are calves. The measles persist for more than 2 years. Consequently, sanitary measures for prevention of infection with this parasite, which causes losses by condemnation and special processing of carcasses, should be employed not only during calfhood, but also until the cattle go to market. (ADP b1-28)

H. Winter Coccidiosis (Bloody Scours) of Cattle.

Studies on this project were continued at the ADP Regional Animal Disease Laboratory at Logan, Utah. Washed sporulated oocysts of Eimeria bovis were injected either intraperitoneally, intravenously or subcutaneously into young calves in an attempt to establish an immune response. No reactions occurred in any of the calves as a result of the injections. No intestinal infections with coccidia occurred. Six weeks after these injections, oral inoculations with E. bovis oocysts were given to these calves plus a group of previously uninjected control calves. Coccidial infections developed in all four groups of calves indicating an absence of immunity in those previously injected with oocysts. Upon recovery from infection, the calves were divided into 3 groups. One group received an intramuscular injection of hormone (ACTH), and another group received an intramuscular injection of cortisone acetate. Later the 3 groups were given oral inoculations of E. bovis oocysts to challenge their immunity. There was no significant reinfection. Immunity apparently develops only as a result of intestinal infection. No changes in the blood serum potassium and sodium levels were detectable until shortly before death of the animals.

In studies to determine the number of oocysts required to produce an active immunity, 3 calves were given daily oral inoculations of 1,000, 5,000, and 15,000 Eimeria bovis sporulated oocysts, respectively, for 47 days. No significant difference was determined in the degree of immunity. Cross inoculation tests showed that severe infection with E. bovis did not protect against infection with E. zurnii.

One of two calves inoculated with sporulated oocysts of Eimeria zurnii, refrigerated at 5°C for more than 2 years, died with severe symptoms. The other calf developed less severe symptoms and survived. Cortisone acetate injected subcutaneously in young calves was used in an attempt to develop a stress method for establishing consistent infections with E. zurnii. Oral inoculations of injected and uninjected control calves resulted in inconsistent infection. (Logan, Utah)

Research workers at the Montana Veterinary Research Laboratory, Agricultural Experiment Station, Bozeman, under a cooperative agreement with the USDA, determined from observations on 8 disease outbreaks in cattle tentatively identified as "winter" coccidiosis, indicated that Eimeria zurnii was the predominant organism occurring in 4 cases, E. bovis in 3, and E. canadensis E. brasiliensis each in 1 case. Final diagnosis of clinical coccidiosis was made in only 3 instances in which E. zurnii occurred alone. A severe

case of bloody diarrhea was observed in a mature cow in which the ciliate protozoan Buxtonella sulcata apparently was the causative agent. (Montana) (ADP b1-29)

I. Anaplasmosis of Cattle.

At the Beltsville Parasitological Laboratory, research workers reported the following findings: Ten trials to determine if females of the tick, Dermacentor andersoni, can transmit Anaplasma marginale "hereditarily," or not, were conducted using splenectomized calves. All of these trials proved negative for this type of transmission of the anaplasm from adult to larval ticks.

Agar-gel double diffusion studies revealed that precipitating antibodies do occur naturally in the serums of cattle affected with anaplasmosis. Complement-fixing antigen served as the precipitating antigen in these studies.

Electron microscopy and immunofluorescent studies have indicated that all but one isolate of A. marginale, thus far studied, have appendages which cannot be seen with conventional microscopes. This appendage assists in the identification of the parasite in preparations made from infected ticks.

A commercial, chemically purified isomer of fluorescein isothiocyanate was found to yield optimum brilliance when conjugated to immune bovine serum, which was then used to stain blood films containing A. marginale.

The resistance of carriers of A. marginale to re-exposure with the same agent was studied. It was found that some carriers withstood re-exposure, while others developed acute signs of infection.

Epizootiological studies over a 6-year period in a dairy herd in southern Louisiana have shown that acute anaplasmosis caused a 26 percent loss in milk production in lactating cows. There was no appreciable decrease in milk production of infected cows during the carrier stage.

A beef herd at the Kerrville Station, Kerrville, Texas, which in 1958 was heavily infected with anaplasmosis, has now become free of the infection. Clean heifers were used to replace their infected dams. During this year, the few animals remaining in the "reactor" herd were removed. Complement-fixation tests made at weaning time last August showed 7 reactors and 11 negative in 18 calves from the reactor cows. The 8 calves from the cows in the negative component were negative. During the February 19, 1963, complement-fixation testing, 60 days after vector peaking, one 2-year-old heifer was found to have a positive (1:10) titer. She was removed from the herd, kept in isolation and periodically retested. At the end of fiscal year 1963, she continues to give a "doubtful" reaction. At this time the

herd consists of 1 bull, 37 cows and heifers 2 to 4 years old, 9 yearling and 20 calves. A total of 383 blood samples were obtained and processed for complement-fixation testing. (Kerrville, Texas) (ADP b1-30)

Under a PL 480 grant to the School of Veterinary, Montevideo, Uruguay, research was conduted on anaplasmosis, piroplasmosis, and Babesialosis of cattle. The report of the work shows considerable progress has been made.

Bovine erythrocytes, some containing Anaplasma, and some containing Babesia, were washed and inoculated into cultures of both swine kidney cells and Hela cells. Growth of the parasites was not observed. Cellular deterioration occurred within 5 days. Hela cells were affected to a lesser degree than swine kidney cells. Cultures of splenic tissue of rats did not deteriorate following addition of erythrocytes from bovines in the clinical phase of anaplasmosis.

Electrophoretic studies were made of the serums of many experimentally anaplasmosis-infected bovines. Before anaplasms were detected in the peripheral blood, there was a decrease in the concentration of total serum proteins (TSP), of albumin, and in the albumin/globulin (AG) ratio, and a slight decrease in gamma globulin. The time when anaplasms were most numerous corresponded to the lowest level of TSP, albumin, gamma globulin, and of the albumin/globulin ratio.

Alpha and beta globulins increased during the entire course of the clinical disease, reaching their highest levels at end, 43 days, and then diminishing to pre-infection levels. The concentration of gamma globulin increased as the level of the alpha and beta globulins returned to normal. The concentration of gamma globulin was highest at the end of the study, 65 days after infection. The concentration of antibodies appears to be greater in the alpha and beta globulins than in the gamma fraction. Changes were not observed in control animals.

Anaplasmosis-experimentally-infected bovines were inoculated, intravenously, with 2 doses of Spirotripan, 20 cc per dose. Some animals were inoculated during the time the anaplasms in the blood were on the increase; others were treated during the height of the infection. These treatments were ineffectual in altering materially the natural course of the infection or of the disease.

The "mechanism of anemia" in anaplasmosis was studied in splenectomized rats, experimentally infected with Anaplasma ratti. A transient anemia, of a few days duration, was observed. Intracellular bodies in erythrocytes of 2 animals were observed. The bodies bore some resemblance to anaplasms, but could not be positively identified.

Tissue culture techniques were used to produce ovarian, muscular, and "glandular" cells of the tick Boophilus microplus for use as a media in the study of developmental stages of Babesia and Anaplasma. Only "a survival

phenomenon" occurred and, in some cultures of ovarian tissues, limited development of the ovocites. (Uruguay) (S9-ADP-1)

J. Parasites of Cattle - Stephanofilarial Species.

This is a preliminary report on a new project initiated at the ADP Regional Animal Disease Laboratory, University Park, New Mexico. It concerns studies of worm parasites of cattle on irrigated pastures and on high-rainfall areas of the Southwest, with special emphasis on the Stephanofilarial species. Stephanofilaria stilesi has been found in 26 of 28 (93%) beef cattle examined. The lesions caused by this nematode were usually restricted to the region of the brisket in young animals and were from 1 to 2 inches in diameter, but in older cattle the lesions often extended from the brisket to the udder or scrotum, involving as much as 2 square feet of skin. Ten of 25 (40%) dairy animals examined also had lesions typical of the disease. As in beef cattle, the udder was often involved. A study of the mode of transmission of the parasite is in progress. (New Mexico) (ADP b1-33)

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AREA NO. 12 - PARASITES AND PARASITIC DISEASES OF SWINE

Problem. Parasitic diseases have been estimated to cost the swine industry of the United States at least $200 million annually. These diseases for the most part are cosmopolitan. Subclinical infections are the most frequent type and the most costly, yet they are generally so difficult to recognize that they often are overlooked entirely. Diagnosis is difficult, and successful treatments for many of these parasitisms are not available. Moreover, management practices to avoid the spread of parasitisms and to control them are often ineffectual. The problem is to develop, through a planned, balanced program of basic and applied research, knowledge for preventing, controlling, or eradicating parasitic diseases so as to provide for healthy swine, insure adequate supplies of parasite-free pork for an expanding population, avoid or minimize economic losses caused by these diseases, and thereby contribute to a prosperous agriculture, a sound national economy, a high standard of living, and a healthy population.

USDA PROGRAM

The Department has a continuing long-term program involving parasitologists, veterinarians, biochemists, microbiologists, and pathologists engaged in basic and applied research in this problem area. Research is being conducted on the following diseases at the designated locations.

The Federal scientific effort devoted to research in this area totals 4.7 professional man years. This effort is divided among sub-headings as follows:

The role of parasites in the economy of swine production 1.2 at the Beltsville Parasitological Laboratory, Beltsville, Maryland, and at the Divisions laboratory at Tifton, Georgia, through informal cooperation with the Georgia Coastal Plain Experiment Station.

Bionomics and pathogenicity of the swine whipworm 0.5 at the Beltsville Parasitological Laboratory.

Swine kidney worms 2.1 at Tifton, Georgia, the Beltsville Parasitological Laboratory, and under cooperative agreement with the North Carolina Agricultural Experiment Station at Raleigh.

Investigations of Trichinella spiralis 0.5 at the Beltsville Parasitological Laboratory.

Effect of anthelmintic treatment on rate of gain 0.3 at Tifton, Georgia.

Pathogenic role of the intestinal roundworm 0.1 under a cooperative agreement with the Nebraska Agricultural Experiment Station at Lincoln.

REPORT OF PROGRESS FOR USDA AND COOPERATIVE PROGRAMS

A. Swine kidney worms.

Past research results have shown that the swine kidneyworm, Stephanurus dentatus, can be completely eradicated from an infested area through herd management. The management system consists of using only gilts for breeders and removing all older animals from the farm as well as the breeders after pigs are weaned.

Application of the "gilt only" system of herd management now in progress under farm conditions seems to support the research results - that kidneyworm can be eradicated from infested farms in 2 years or less.

At the North Carolina Agricultural Experiment Station, several organophosphate compounds were given to kidneyworm-infested swine to determine their influence on ova production. None of the compounds decreased ova production when used at a safe dosage level. Studies were continued to determine if prenatal infection with swine kidneyworms occurs. (ADP b2-11(R)

B. Intestinal threadworms.

The intestinal threadworm, Strongyloides ransomi, causes heavy losses in baby pigs in the North Florida and South Georgia area. It has now been demonstrated that S. ransomi infection of weaned pigs can reduce the weight gain and efficiency of pigs. The extra feed necessary for an infected pig to reach market weight may cost as much as $3.70 more than that for an uninfected animal. (ADP b2-17)

C. Intestinal roundworms.

At the Nebraska Agricultural Experiment Station, Lincoln, immunity to migrating Ascaris suum was induced in pigs by administering three oral inoculations of ascaris eggs at 10-day intervals. Fifteen days following the last immunizing dose, the pigs were challenged with 100,000 infective eggs. Pigs, receiving their first inoculations at 2 weeks of age, developed a poor immunity. Pigs placed on trial at 4 months of age (and previously infected with ascaris) had the best immunity.

CoRal at 20 parts per million continuously in diet of growing-finishing swine did not depress Ascaris infections. It inhibited fly development in the feces of the treated pigs.

Oil suspensions of thiabendazole stopped the migratory stage of Ascaris in baby pigs but have a short residual effect. (ADP b2-12(Rev.)

D. PL 480 Project.

1. Investigations on Trichinellosis with special reference to epizootiology, immunology and pathogenesis.

Investigations are being conducted at the Polish Academy of Science, Warsaw, on the epidemiological, epizootiological, and immunological aspects of trichinosis. Arrangements have been made with 6 sources of human autopsy material for the examination of tissues for trichinae. Forty-five samples of such tissues have already been examined. Microscopic and digestion techniques are used for these examinations. Studies of the incidence of trichinae in domestic dogs and cats, and in wild carnivores (foxes, etc.), are also being carried out. Seventy-five specimens from these sources have been examined. Pigs, rabbits and mice have been experimentally infected with trichinae and will be used to develop an antigen for use in the laboratory and field diagnosis of trichinosis. (E21-ADP-9)

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AREA NO. 13 - PARASITES AND PARASITIC DISEASES OF SHEEP AND GOATS

Problem. The cost of parasitic diseases to the sheep and goat industry of the United States is estimated to be in excess of $45 million, annually. Disorders caused by parasites are ubiquitous, generally insidious and often overlooked entirely. Diagnosis is difficult, and successful treatments for many of these diseases are not available. Moreover, management practices to avoid spread of parasitisms and to control them are often ineffectual. The problem is to develop, through a planned, balanced program of basic and applied research, knowledge for preventing, controlling or eradicating parasitic diseases so as to provide for healthy animals, insure adequate supplies of high quality lamb for an expanding population, avoid or minimize economic losses caused by these diseases, and thereby contribute to a prosperous agriculture, a sound national economy, a high standard of living, and a healthy population.

USDA PROGRAM

The Department has a continuous long-term program involving biochemists, parasitologists, and veterinarians engaged in both basic studies and the application of known principles to the solution of parasites and parasitic diseases of sheep and goats. Research is being conducted on these diseases at the designated locations.

The Federal scientific effort devoted to research in this area totals 8.2 professional man-years. This effort is divided among sub-headings as follows:

Lungworms 1.0 at the Beltsville Parasitological Laboratory.

Bionomics of Coccidial Parasites 2.0 at the Beltsville Parasitological Laboratory.

Effects of Helminth Infections on Serum Proteins 0.5 at the Beltsville Parasitological Laboratory.

Gastrointestinal Nematodes 2.1 at the Beltsville Parasitological Laboratory, and under a cooperative agreement with the Kentucky Agricultural Experiment Station at Lexington.

Helminth and Protozoan Parasitism in the South 1.5 at the Regional Animal Disease Research Laboratory, Auburn, Alabama, and through informal cooperation with the Mississippi Agricultural Experiment Station, State College.

Biology, Pathogenesis, and Control of Helminth Parasites of Sheep in the Southwest 1.0 at the University Park, New Mexico, field station, and through informal cooperation with the New Mexico Agricultural Experiment Station at University Park.

Effect of Intestinal Roundworms on Metabolism 0.1 under cooperative agreement with the North Dakota Agricultural Experiment Station, Fargo.

REPORT OF PROGRESS FOR USDA AND COOPERATIVE PROGRAMS

A. Effects of Helminth Infections on Serum Proteins.

The serum proteins of three groups of 4 Shropshire lambs were analyzed by a paper electrophoresis technique to investigate differences which might result from parasitism or management practices, or both. Serums from lambs raised helminth-free had consistently higher albumin/globulin ratios (A/G) and lower gamma globulin percentages than did serums from lightly parasitized, barn-raised lambs.

Serums from 10-month-old barn-raised lambs had a gamma globulin average of 16 percent and an average A/G ratio of 2.2, whereas serums from naturally parasitized lambs on pasture had a gamma globulin average of 46 percent and an A/G ratio of 0.4. Total serum protein values were higher in barn-raised lambs than in either of the other two groups.

The A/G ratios and total serum protein values rose as the lambs matured, when significant parasitic infections were absent, but decreased with age in parasitized animals.

Inhibited development of Haemonchus contortus larvae apparently occurred in one lamb. This inhibited development may be related to the high gamma globulin percentage produced by previous exposure to this nematode. (Beltsville, Maryland) (ADP b3-15)

B. Gastrointestinal Nematodes.

Preliminary studies of the mechanism of control exerted by the immunological response of the sheep host on the developmental stages and fecundity of the intestinal thread-necked worm, Nematodirus spathiger, have been completed. The results of these studies show that control of the parasitic infection is centered about threshold levels of immunological responsiveness and is expressed by (1) elimination at the infective, or 3rd larval, stage, (2) retardation of development at the 4th larval stage, (3) reduction in egg production of the adult female, (4) elimination of adult worms, and (5) discrimination against the female in both the degree of retardation of the 4th larval stage and the extent of elimination in the adult stage. Information such as this is required to better understand the relationship between host and parasite in order that more effective control measures against ovine parasites can be devised. (Beltsville, Md.)

In cooperation with the University of Kentucky, in a comparative evaluation in lambs, a series of six monthly drenches of thiabendazole, ruelene, or phenothiazine during a summer grazing season doubled the rate of gain of body weight over that of a control group. At the close of the test the body

weights averaged 92.2 pounds for the thiabendazole-treated group, 95.3 pounds for the ruelene-treated group, 97.7 pounds for the phenothiazine-treated group, and 79.6 pounds for the untreated group. The relatively poor performance of the thiabendazole-treated group was due to the selection and build-up during the course of the study of a population of the common stomach worm that was not effectively controlled by the 50 mg/kg dose of thiabendazole. Two of the 10 lambs in the untreated group died of acute parasitism. One of the ruelene-treated group died of an undetermined cause. (Lexington, Kentucky) (ADP b3-16)

C. Helminth and Protozoan Parasitism in the South.

The life cycle of Eimeria ahsata in sheep has been worked out at the Regional Laboratory, Auburn, Alabama. The prepatent period varies from 18 to 21 days. Sporozoites were found in the intestinal mucosa at 2 and 5 days. Schizonts measuring 50 μ were found by the 10th day and the largest measuring 162 by 265 μ was seen on the 15th day. It is suggested that only one generation of schizonts and merozoites is produced. Gametogenesis was first seen at 11 days with oocysts located mostly in cells lining the intestinal glands. The endogenous stages were located throughout the small intestine with the greatest numbers in the jejunum. (Auburn, Alabama) (ADP b3-13:b3-19)

D. Biology, Pathogenesis, and Control of Helminth Parasites of Sheep in the Southwest.

Efforts were made to demonstrate functional immunity against haemonchosis in lambs under both field and laboratory conditions by inoculating them with a naturally attenuated strain of the parasite from pronghorn antelope and administering an anthelmintic 21 days later to remove the worms. A total of 39 worm-free lambs were used. As compared with untreated inoculated groups, lambs treated as described above showed no evidence of an immunity. Apparently removal of the infections by means of an anthelmintic 21 days after inoculation resulted in the loss of the immunizing effect. Further work with the attenuated strain as a possible immunizing agent is warranted.

Liver flukes were found to be economically important parasites in sheep in one additional area in New Mexico and in one area in Colorado. A total of 38 sheep from 3 different farms were examined and over 50 percent were infected. Fossaria modicella, a snail known to be a vector in other areas of the West, was found on two of the farms in question, but it remains to be determined whether this snail is involved in the transmission of flukes in New Mexico and southern Colorado.

Information as to the occurrence of lungworms in sheep was obtained from southern Colorado. Thirteen of 26 animals examined harbored the parasites. Information about this occurrence and about a case in New Mexico sheep was assembled and described in a report now in press. This constitutes the first record of lungworm in sheep in the two States concerned.

Controlled anthelmintic tests showed Hetol at a dose rate of 8.6 g/sheep to be 100 percent effective in removing adult common liver flukes from sheep, but ineffective against immature (half grown and under) flukes and against the liver tapeworm. Similar tests involving Bayer 2353 and Bayer ME3625 and sheep harboring the liver tapeworm demonstrated that the former compound in particular, when given at a dose rate of about 500 mg/kg, is sufficiently promising to warrant further investigation. (University Park, New Mexico) (ADP b3-18)

E. Effect of Intestinal Roundworms on Metabolism

Under a cooperative agreement with the North Dakota Agricultural Experiment Station, work was continued,in a study of the effects of gastrointestinal parasitism, on the sulfur content and tensile strength of wool. Relatively parasite-free lambs as nearly identical as possible were separated into three groups. One group was kept as noninfected controls; one group was given 5,000 infective larvae of gastrointestinal nematodes per lamb, and the third group was given 50,000 infective larvae per lamb. Wool samples and blood samples were collected every two weeks during the study. Tensile strength was apparently adversely affected in the 50,000 larvae/lamb group but not in the 5,000 larvae/lamb group. All groups showed a gain in sulfur content of wool during the study, but the control showed the greatest gain and the 50,000 larvae/lamb group showed the least gain in sulfur content of the wool. (Fargo, North Dakota) (ADP b3-7(R)

PUBLICATIONS REPORTING RESULTS OF USDA AND COOPERATIVE RESEARCH

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_____ and G. I. Wilson. 1962. Serum protein studies on sheep and goats. I. Studies on Shropshire lambs exposed to different degrees of parasitism. Amer. J. Vet. Res., 23(95): 718-724.

AREA NO. 14 - PARASITES AND PARASITIC DISEASES OF POULTRY

Problem. Parasites and parasitic diseases probably cost the poultry industry many millions of dollars annually by causing intestinal disturbances, emaciation, retarded growth, reduced egg production, and deaths. Parasites are ubiquitous, many times insidious, and often overlooked until birds are damaged irreparably. Early diagnosis is difficult, and reliable treatments for many devastating parasitoses are not available. Moreover, some management practices, intended to avoid spread of parasites and to control them, have been found ineffectual as is shown by the increasing importance of certain parasites in broiler production. The problem is to develop, through a planned, balanced program of basic and applied research, methods for preventing, controlling or eradicating parasitic diseases, thus affording economical production of healthy poultry and sound products in supplies adequate to meet the needs of an expanding population.

USDA PROGRAM

The Department has a continuous long-term program involving parasitologists, biologists, and chemists, engaged in both basic studies and the application of known principles to the solution of the problem of parasites and parasitic diseases of poultry.

The Federal scientific effort devoted to research in this area totals 5.5 professional man-years. This effort is applied as follows:

Bionomics of Intestinal Protozoan Parasites 0.5 at the Beltsville Parasitological Laboratory, Beltsville, Maryland.

Immunology of Protozoan Parasitic Diseases 1.5 at the Beltsville Parasitological Laboratory, Beltsville, Maryland.

Control of Coccidiosis 2.0 at the Beltsville Parasitological Laboratory, Beltsville, Maryland.

Biology of Nematode Parasites 1.5 at the Beltsville Parasitological Laboratory, Beltsville, Maryland.

REPORT OF PROGRESS FOR USDA AND COOPERATIVE PROGRAMS

A. Control of Coccidiosis.

Studies at the Beltsville Parasitological Laboratory (BPL) have shown that Eimeria acervulina oocysts in potassium dichromate produced 58% excystation after storage for 318 days in a refrigerator in which the temperature dropped below freezing for an undetermined interval. Oocysts from the same culture,

however, did not produce infection in 4-week-old chickens. The sporozoites that excysted in vitro were in poor condition and, contrary to the usual case, did not survive long. These findings indicate that sporozoites can excyst and be non-infective.

Earlier studies showed that 3-day-old chicks produced 13 times more oocysts than did 1-day-old birds when both age groups were given similar dosages of sporulated oocysts. Investigations of this marked difference in oocyst yield revealed that the following factors may be involved: (1) amount of development of the gizzard mucosa and musculature; (2) intestinal concentrations of trypsin and chymotrypsin--pancreatic enzymes involved in the excystation process--in the vicinity of the sporocysts; (3) amount of yolk sac material in the intestine, and (4) amount of development of the intestinal villi.

In continuing studies, chicks were raised to 7 to 8 days of age with bacterial populations in their intestines sufficiently reduced so that cultures of duodenal tissue could be prepared using only minimal amounts of antibiotics. During the year, 24 chicks were hatched and raised by a very simple and inexpensive method. There was no contamination of duodenal tissue cultures.

A Trithiadol-fast strain of Eimeria tenella was developed experimentally at BPL. It proved resistant to the field level of Trithiadol recommended by the manufacturers and, in addition, exhibited a degree of tolerance to nitrofurazone, Unistat, and Zoalene.

Nihydrazone, a recently introduced nitrofuran compound, was tested for effectiveness against cecal coccidiosis (Eimeria tenella). It proved effective in preventing mortality and reduced the severity of cecal lesions to a significant degree; growth of infected nihydrazone-medicated birds, however, was poor. A similar level of medication did not adversely affect the growth of uninfected birds.

Various combinations of chlortetracycline, sulfaquinoxaline, and terephthalic acid were included in the diet of birds infected with cecal coccidiosis. Low-level chlortetracycline (0.0055%) was potentiated to some extent by 0.5% terephthalic acid, but not sufficiently to be commercially practical. Effectiveness of the prophylactic level of sulfaquinoxaline (0.0125%) was increased by the addition of chlortetracycline, as was already known.

Mutual cross-resistance was noted between a nitrofurazone-resistant and a zoalene-resistant strain of Eimeria tenella. At the same time, the nitrofurazone-resistant strain did not prove to be resistant to glycarbylamide or Trithiadol, even though strains resistant to these compounds were also cross-resistant to nitrofurazone. (ADP b4-9)

B. Biology of Nematode Parasites.

Research at BPL has shown that the processes by which the infective agents (sporozoites) are released or "excyst" from oocysts of chicken and turkey

coccidia are similar. Two species, Eimeria acervulina of chickens and E. meleagrimitis of turkeys, which live in the upper small intestine, excyst rapidly in the region that they infect, while two species, E. tenella of chickens, and E. gallopavonis of turkeys, require more time for excystation and excyst in the middle and lower intestinal tract close to the tissues that they infect. (ADP b4-11)

PUBLICATIONS REPORTING RESULTS OF USDA RESEARCH

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AREA NO. 15 - TREATMENT FOR REMOVAL OF PARASITES OF DOMESTIC ANIMALS

Problem. Parasites of food animals are responsible for losses to livestock producers approximating a billion dollars annually. This estimate, moreover, is conservative since it does not take into account costs of treatment and other control measures. Chemical antiparasitic agents are the most powerful weapons presently available against parasites and the diseases they cause, yet specific treatments generally have a comparatively short period of usefulness. Many of the currently preferred treatments were unknown a decade or so ago and, in all probability few, if any of those in use today will be primary choices a decade or so hence. Moreover, the growing concern with respect to residues in edible tissues and organs of treated animals and birds necessitates development of control measures other than treatment. The problem is to develop, through a planned, balanced program of basic and applied research control methods that minimize reliance on extrinsic chemicals. These include investigations of immunological procedures, management practices which minimize exposure of animals to parasitic infections, and natural control agents such as parasites, pathogenic microorganisms, and predators of economically important livestock pests.

USDA PROGRAM

The Department has a continuing long-term program involving veterinarians, parasitologists, pharmacologists, and biochemists engaged in both basic studies and the application of known principles in developing treatments for removal or control of parasites of domestic animals. Research is being conducted on this problem at the following designated locations.

The Federal scientific effort devoted to research in this area totals 13.0 professional man-years. This effort is applied as follows:

Chemical Control of Parasitic Diseases 1.5 at the Beltsville Parasitological Laboratory, Beltsville, Maryland.

New and Improved Anthelmintics 3.0 at the Beltsville Parasitological Laboratory, Beltsville, Maryland.

Hazards of Residues from Treatment for Parasites 3.5 at the Regional Animal Disease Research Laboratory, Auburn, Alabama.

Parasitic and Related Skin Diseases 1.5 at the Albuquerque, New Mexico, field station.

Pathobiology of Parasitic Infections 1.0 at the Albuquerque, New Mexico, field station.

Methods for Control and Eradication of Ticks 1.0 at the Albuquerque, New Mexico, field station.

Control and Eradication of Scabies 1.5 at the Albuquerque, New Mexico, field station.

REPORT OF PROGRESS FOR USDA AND COOPERATIVE PROGRAMS

A. Chemical Control of Parasitic Diseases

Studies at the Beltsville Parasitological Laboratory have shown that imidazole derivatives, metronidazole, and dimetridazole, were each successfully used to eliminate Trichomonas gallinae from naturally infected pigeons in limited trials. This parasite is the causative agent of upper digestive tract trichomoniasis in turkeys, game birds, and pigeons. (ADP b5-5)

In experiments at Auburn, Alabama, thiabendazole, given to calves at a dose rate of 55 mg/kg, was highly effective (93-98%) against fourth-stage Trichostrongylus axei and fourth-molt-stage Cooperia pectinata, and Trichostrongylus colubriformis. It was ineffective against fourth-stage Ostertagia ostertagi. The drug was moderately effective (76-88%) against third-stage T. axei, C. oncophora, and T. colubriformis, and only 42% effective against third-stage O. ostertagi. (ADP b5-6)

B. New and Improved Anthelmintics

In work conducted at the Beltsville Parasitological Laboratory, Trichomonas foetus, the causative agent of one of the major reproductive diseases of cattle, has been found to be refractory to efficient systemic therapy. However, in limited trials, six bulls with experimental T. foetus infections have been treated effectively with dimetridazole administered orally. Trichomonads have not been recovered from these animals on post-treatment examinations made over periods of one to six months. (ADP b5-9)

C. Parasitic and Related Skin Diseases

At Albuquerque, New Mexico, work has been in progress on antiparasitic agents and measures for the control of parasites belonging to the family Oestridae. This work, now being reported, was begun in the fall of 1961, and concluded in the spring of 1963.

A herd of 3,000 Herefords in Virginia, where two species of cattle grubs exist, was sprayed with Co-Ral, in dilute (0.25%) suspension, once only in the early fall of 1961, and again precisely a year later. As a result of these treatments, which involved the use of a walk-through spray apparatus, cattle grubs were controlled at a remarkably high level. Overall cattle grub population reduction in this unisolated herd, at the end of the second year, approached 99%, when compared with infestations in untreated cattle on 10 neighboring farms.

The treatment, which has the added virtue of controlling mites, lice and ticks, appears acceptable from a variety of standpoints for control of grubs on a large-scale or area-wide basis, a subject of immediate interest to the cattle industry.

Tests Conducted in New Mexico. In light of the extremely high order of effectiveness of Co-Ral (0.25% suspension), applied by means of a walk-through spraying device, against ox-warbles of cattle in Virginia, as described above, replication of this method of control in a dissimilar climatic environment was considered advisable. During September, 1962, New Mexico range cattle with a history of heavy grub infestations, were treated in a manner identical to that employed in Virginia. Results, in terms of grub destruction after treatment for one year only, were virtually the same in the two localities.

A second consideration in conducting the small-scale test in New Mexico was to evaluate the contribution made by the "grub-rake" to walk-through spraying. Co-Ral applied to New Mexico cattle in the Spray-Dip device, both with and without accompanying use of the "grub-rake" was 90-95% effective against ox-warbles. Spraying with the rake appeared to be slightly better than treatment without it, based on the criterion of completely eliminating grubs from the cattle.

Use of the rake is recommended in any community program aimed at the rapid elimination of cattle grubs from all cattle within the treatment zone.

Over a five-year period, an attempt was made to eliminate Oestrus ovis from an isolated flock of New Mexico range sheep, which involved 700-800 breeding ewes. Four annual treatments of the entire flock, using Dimethoate, were scheduled. However, consecutive treatments of all animals were possible only during the last two years. The dose varied from 14 to 24 mg/kg intramuscularly (IM). A high degree of control was achieved as the average number of larvae dropped from 49.8 in 1958 to 8.7 in 1962. During the same period, larval populations in control sheep from comparable areas increased from 30.9 in 1958 to 68.2 in 1962.

Dimethoate Toxicity to Sheep. Previous annual reports submitted by this laboratory have detailed the toxicity of Dimethoate to sheep when given intramuscularly. A reappraisal of this problem has recently been made. Dimethoate is safe to use at 25 mg/kg only when the material is fresh. When over a year old, 19 mg/kg should be the top dose given to range sheep, and when over two years old, the material should not be administered at more than 14 mg/kg.

Dimethoate 45% IM, from two different lots, varying in shelf age from 2 to 26 months, was administered to 4,675 sheep at dosages ranging from 14 to 32 mg/kg. There appeared to be a direct relationship between the shelf age of the product and the degree of its toxicity to sheep.

Famophis 35% IM was administered to 43 sheep at dosages ranging from 10 to 70 mg/kg and proved disappointing in its effectiveness against second and third stage larvae of O. ovis. It did not exhibit toxicity to sheep at any of the doses tried. (ADP b5-17)

In work at Albuquerque on the problem of mange, examinations of sheep revealed the presence of the leg mange mite in flocks from each of 8 States involved in a survey concluded during the summer of 1962. The States involved were Arizona, California, Colorado, Iowa, New Mexico, Oklahoma, Texas, and Virginia. Fifty-seven percent of the flocks or assembled lots of animals were positive for presence of the parasite.

The leg mange mite was found more frequently in small farm flocks than on ranches, and was encountered with greater frequency than any other orthropod parasite, including the sheep ked, the foot louse, the biting or little red louse, and the spinose ear tick. On the basis of this preliminary investigation, the leg mange mite may very likely be a common inhabitant of the lower extremities of sheep over much, if not all, of the continental United States. (ADP b5-12)

D. Pathobiology of Parasitic Infections.

Investigations by scientists at the Albuquerque laboratory have continued. In 1957, an effort to eliminate lice from a herd of 3,000 Herefords in Virginia was begun. The study was concluded during the spring of 1963. Control measures involved spraying of all herd members, on a systematic, yet commercially acceptable basis, with effective, FDA-approved lousicides. Of the four species of lice with which the herd was originally infested, one was judged eliminated as a result of treatment. A second species was not found at the conclusion of the study, but its absence was attributed in part to natural factors, and not wholly to treatment; its return to the herd is considered likely. Two species of lice remained. Only one of these, the calf louse, persisted in maintaining an infestation at high frequency level, yet with populations so small as to be of no economic significance.

Of all treatment regimens employed, the use of Malathion, applied twice in two weeks, was the most successful. The value of continued search for simple techniques and effective drugs, suitable for eliminating lice from farm and ranch herds, is clearly emphasized by this study. (ADP b5-13)

PUBLICATIONS REPORTING RESULTS OF USDA RESEARCH

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Ciordia, H., W. E. Bizzell, D. M. Baird, and H. C. McCampbell. 1963. Effect of weaning age and creep rations on parasitism in lambs. Jour. Anim. Sci., 22: 237.

Colbenson, H. P. 1962. Spinose Ear Tick, Ornithodoros (Otobius) megnini, from Pronghorn Antelope. J. Parasit., 48(4) August. 630.

Hanosh, G. J., and Irwin H. Roberts. 1963. A Portable Table for Examining Small Ruminants. Amer. J. Vet. Res., 24: 220-222. January.

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Roberts, Irwin H., and H. P. Colbenson. 1963. Larvae of Oestrus ovis in the ears of a sheep. Amer. J. Vet. Res., 24: 628-630. May.

AREA 16 - MISCELLANEOUS PARASITES AND PARASITIC DISEASES

Problem. Parasitism is a way of life that characterizes the majority of living things. Except for basic life processes, it is probably the commonest biological phenomenon. More than 50,000 kinds of animal parasites (i.e., parasites classified as animals as opposed to those classified as plants) are known. New varieties are being discovered and described at a rate of about 500 per year. Some devastating parasites, indigenous to foreign countries, threaten to surmount barriers imposed against them. Certain of these have already gained new footholds in livestock, poultry, and wildlife. Essential elements of procedure against parasites--established, exotic, or new--are accurate diagnosis, development of full knowledge about them, and research on effective control measures. The primary requirement is development through research of up-to-date knowledge of classification and identification supported by a complete reference collection of parasites, including type specimens and familiarity with global research already done. Basic investigations of parasitisms as biological phenomena are involved, especially in host-parasite relations, immunology, serology, ultrastructure, and other aspects of diagnosis and control. The problem is to develop and maintain up-to-date methods of identification and the essential, supporting reference collections, as well as complete parasitological information extracted from the world's scientific literature; investigate important phenomena and host-parasite systems not covered in specific host categories; and provide bases for detection and control that are adequate to meet existing and anticipated needs, through research on problems involving various parasites and hosts, including wild animals and birds important to agriculture.

USDA PROGRAM

The Department has a continuing long-term program for parasitologists, biochemists, and microbiologists, engaged in basic and applied research in this area. Research is being conducted on the following problems at the designated locations.

The Federal scientific effort devoted to research in this area totals 10.5 professional man years. This effort is divided among subheadings as follows:

Classification of parasites 2.0 at the Beltsville Parasitological Laboratory.

Maintenance of parasite collection 1.0 at the Beltsville Parasitological Laboratory.

Maintenance and publication of author, subject, and host index-catalogues 2.5 at the Beltsville Parasitological Laboratory.

Immunologic and other biologic approaches to the prevention and control of parasitic diseases 5.0 at the Beltsville Parasitological Laboratory.

REPORT OF PROGRESS FOR USDA PROGRAMS

A. Maintenance and publication of author, subject, and host index-catalogues. The Index-Catalogue of Medical and Veterinary Zoology has been maintained and expanded in its various sections: Author, Subject, and Host Catalogues, and Checklist of Specific and Subspecific Names. New entries augmenting the Catalogues are as follows: Author entries, 7,505; Parasite entries, 15,850; and Host entries, 12,330. The Index Catalogue has continued to supply references for the Treatment Catalogue of the Antiparasitic Investigations Research Group and to index literature on plant parasitic nematodes. New genera and species of parasites are as follows: Protozoa: 5 n.g., 178 n.sp.; Trematoda: 22 n.g., 189 n.sp.; Cestoda: 10 n.g., 41 n.sp.; Nematoda: 11 n.g., 167 n.sp.; Arthropoda and miscellaneous groups: 26 n.g., 373 n.sp.; Total, 74 n.g., 948 n.sp.

There have been 224 new citations of periodicals added to the Catalogue. An average of 500 periodicals are examined each day at the National Agricultural Library for parasitological papers to be indexed in the Index-Catalogue.

The Index-Catalogue has had approximately 133 visitors from the United States and 18 other countries, consulting it as a source of information. (ADP b6-5(Rev.)

B. Immunologic and other biologic approaches to the prevention and control of parasitic diseases. Antibodies were demonstrated in the pooled sera of pigs infected with Stephanurus dentatus by using living in vitro-grown parasitic larvae of this nematode as sources of antigen. Larvae in third- and fourth-stages showed refractive precipitates at the body openings. Heavy particulate precipitates were also observed free in the serum.

Studies on the in vitro cultivation of the parasitic stages of Stephanurus dentatus in media (Pitts' and SM-1) previously described were continued. No further advancement in development beyond the fourth-stage was obtained in these media. However, the addition of various cell-free or cellular supplements resulted in increased yields of fourth-stage larvae, advanced development within fourth-stage, and better survival. Although some development occurred in chemically defined media, the addition of undefined complexes was necessary for development to fourth-stage.

Adult Stephanurus dentatus have been maintained in simple media containing serum, or in serum alone, for 2 to 4 weeks. Survival in the absence of serum is poor. During survival, the worms produced antigens which were specific for antibodies in sera of swine infected with this nematode. Yields of antigen obtained in this manner are potentially greater than those obtained previously from extracts of whole worms or tissues.

By previously described procedures using living in vitro-grown parasitic larvae of Oesophagostomum radiatum as sources of antigen and the occurrence of two precipitate reactions as visible evidence, antibodies were demonstrated in: 1) serum and extracts of intestinal tissue obtained from a bovine as early as 3 days after infection with this nematode; and 2) serum from a bovine resistant to this nematode for 18 months after the last of 5 challenge doses of infective larvae. There was a positive correlation between the earliest detection of antibodies and the numbers of infective larvae administered in an initial infection. No antibodies to O. radiatum were detected in lymph node extracts from bovines singly infected or resistant to this nematode nor in sera of bovines infected with Cooperia punctata. (ADP b6-10)

1. The Chemical and Physical Elements of Parasites. Apparently swine kidney worm antigens bearing a negative charge at about pH 8.6 are sufficiently specific to warrant their study in a variety of serological procedures with a view to developing a test for the diagnosis of swine kidney worm infection.

Particles present in the regular anaplasmosis antigen were separated into 10 fractions of varying size and/or density by centrifuging them through a sucrose gradient. The fractions were titered, brought to a standard strength, and tested against 10 nonspecific serums. Each fraction gave a reaction with each of the 10 serums, indicating that nonspecific activity occurs in particles of different size and density. (ADP b6-11)

Under a PL 480 Grant to the Institute for Veterinary Research, Pulawy, Poland, for Control of the liver fluke, Fasciola hepatica in domestic ruminants, a determination was made of the efficiency of the following drugs for treatment of the liver fluke (Fasciola hepatica) in cattle - hexachlorethane; hexachlorophene; bistrichloromethylobenzol; and carbon tetrachloride, in mineral oil. The results were generally unsatisfactory, either because of low efficiency or undesirable side effects. (E21-ADP-1)

Under a PL 480 Grant to the Veterinary Faculty, Ankara University, Ankara, Turkey, studies are under way on The Transmission, distribution, and bioeconomics of Fascioliasis (Liver Fluke Disease)of Domestic Animals in Turkey. Hexachlorophene (40-50 mg/kg) killed all 40-day old Fasciola gigantica in sheep. In cattle, 25 mg/kg killed all 50-day old F. gigantica. Fasciola hepatica occurs throughout Turkey, in cattle, sheep, goats, water buffaloes, donkeys, horses, and man.

Snails were collected from many areas. Natural infection of snails with F. hepatica was found in only one species, Lymnaea truncatula. (A22-ADP-1)

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Tromba, F. B. 1962. Immunology of nematode diseases. J. Parasitol. 48: 839-845.

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Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress	Line Proj. Incl. in Area & Sub-Subheading
ADP a1	Infectious and Non-Infectious Diseases of Cattle			
ADP a1-3(R)	Investigations of Brucellosis of Cattle	Ames, Iowa	Yes	1-A
		College Park, Md.	No	
		St. Paul, Minn.	Yes	1-A
		Madison, Wisc.	Yes	1-A
ADP a1-9(R)	Investigations of Vibriosis of Cattle	Ames, Iowa	Yes	1-B
		Ithaca, New York	Yes	1-B
ADP a1-13(R)	Investigations of Tuberculosis of Cattle	Ames, Iowa	No	
		East Lansing, Michigan	Yes	1-C
ADP a1-14C(R)	Mucosal-Respiratory Disease-Complex of Cattle	Ames, Iowa (NADL)	Yes	1-D
		Lafayette, Ind.	Yes	1-D
		Ames, Iowa (Univ.)	Yes	1-D
		Ft. Collins, Colo.	Yes	1-D
ADP a1-15(R)	Investigations of Mastitis of Cattle	Ames, Iowa	Yes	1-E
		Davis, Calif.	Yes	1-E
ADP a1-16(C)*	Treatment of Lead Poisoning in Cattle	St. Paul, Minn.	No	
ADP a1-17	Investigations of Respiratory Diseases of Cattle (Shipping Fever)	Ames, Iowa	No	
ADP a1-18	Investigations of Leptospirosis of Cattle	Ames, Iowa	No	
ADP a1-19	Investigations of Infectious Causes of Infertility in Cattle Other than by Vibriosis and Trichomoniasis	Ames, Iowa	No	
ADP a1-21	Epizootic Bovine Abortion	Ames, Iowa	No	
		Davis, Calif.	Yes	1-F
ADP a1-22	Foot Rot (Infectious Pododermatitis) of Cattle	Ames, Iowa	No	
ADP a1-24	Etiologic, Cytologic, and Histochemic Studies of Pulmonary Adenomatosis in Cattle	Ames, Iowa	No	
ADP a1-35	Investigations of Paratuberculosis (Johne's Disease) of Cattle	Ames, Iowa	Yes	1-G
ADP a1-37**	Investigations of Infectious Keratitis (Pink Eye) of Cattle	Ames, Iowa	No	

*Contract completed during reporting period
**Initiated during reporting period

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Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress	Line Proj. Incl. in Area & Sub-Subheading
ADP a2	Infectious and Non-infectious Diseases of Swine			
ADP a2-8(R)	Studies on the causative agent (or agents), mode of spread, diagnosis, and control of atrophic rhinitis in swine	Ames, Iowa	Yes	2-B
ADP a2-10(R)	Investigations of transmissible gastroenteritis (TGE) complex of young pigs	Ames, Iowa Davis, Calif. Lafayette, Ind.	Yes Yes Yes	2-C 2-C 2-C
ADP a2-13	Pilot field studies to evaluate modified live-virus hog cholera vaccines	Ames, Iowa Live Oak, Florida Valdosta, Georgia	Yes Yes Yes	2-A 2-A 2-A
ADP a2-15	Investigations of erysipelas of swine	Ames, Iowa Pulawy, Poland	Yes Yes	2-D 2-F
ADP a2-16	Investigations of brucellosis of swine	Ames, Iowa	Yes	2-E
ADP a2-17(C)	Investigation of hog cholera	Urbana, Illinois	Yes	2-A
ADP a2-18*	Infectious causes of infertility in swine other than brucellosis and leptospirosis	Ames, Iowa	No	
	*Initiated during reporting period			

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Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress	Line Proj. Incl. in Area & Sub-Subheading
ADP a3	Infectious and Non-Infectious Diseases of Sheep and Goats			
ADP a3-1(R)	Investigations of vibriosis of sheep	Fort Collins, Colo. Bozeman, Montana Logan, Utah	Yes Yes Yes	3-B 3-B 3-B
ADP a3-3	Investigations of scrapie of sheep	Compton, England Edinburgh, Scotland	Yes Yes	3-C 3-C
ADP a3-4	Viral Ulcerative Dermatosis of Sheep	Fort Collins, Colo.	Yes	3-D
ADP a3-5	Investigations of bluetongue in sheep-diagnosis, transmission and control	Denver, Colorado Pullman, Washington	Yes Yes	3-A 3-A
ADP a3-6*	Paratuberculosis (Johne's Disease) of Sheep and Goats	Ames, Iowa	No	
	*Initiated during reporting period			

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Work & Line Project Number	Work and Line Project Titles	Work Location During Past Year	Line Proj. Incl.in Summary of Progress	Line Proj. Incl.in Area & Sub-Subheading
ADP b6	Miscellaneous Parasites and Parasitic Diseases			
ADP b6-13C*	Investigations on the serological, diagnosis, transmission, and control of equine piroplasmosis	Beltsville, Md. Lexington, Ky. Gainesville, Fla	No	
	*Initiated during reporting period			

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Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress	Line Proj. Incl. in Area & Sub-Subheading
ADP a5	Investigations of Infectious and Non-Infectious Diseases of Poultry			
ADP a5-2(R)	Salmonellosis of Poultry	Ames, Iowa	No	
ADP a5-16*	Pasteurellosis of poultry	Ames, Iowa	No	
ADP a5-17	Chronic Respiratory Disease Complex in chickens and turkeys	Ames, Iowa	Yes	5-B
		Storrs, Conn.	Yes	5-B
		Newark, Delaware	Yes	5-B
		Athens, Georgia	Yes	5-B
		Amherst, Mass.	Yes	5-B
		Ithaca, New York	Yes	5-B
		Raleigh, N. C.	Yes	5-B
		College Station, Texas	Yes	5-B
		Blacksburg, Virginia	Yes	5-B
		St. Paul, Minnesota	Yes	5-B
		Hebrew Univ., Israel	No	
ADP a5-18	Newcastle Disease	Athens, Georgia	No	
		Ames, Iowa	No	
		Orono, Maine	Yes	5-C
		Madison, Wisconsin	Yes	5-C
		Pulawy, Poland	Yes	5-C
ADP a5-19(C)	Bluecomb in Turkeys	St. Paul, Minn.	Yes	5-D
ADP a5-20	Ornithosis in Poultry	Davis, Calif.	Yes	5-A
		St. Paul, Minn.	Yes	5-A
		Corvallis, Oregon	Yes	5-A
		College Station, Texas	Yes	5-A
		Ames, Iowa	No	
ADP a5-21	Turkey Airsacculitis	Ames, Iowa	Yes	5-B
		St. Paul, Minn.	Yes	5-B
		Madison, Wisc.	Yes	5-B
ADP a5-22	Study of Avian Leukosis	Ithaca, New York	Yes	5-E

*Transferred to another Area during reporting period.

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Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress	Line Proj. Incl. in Area & Sub-Subheading
ADP a6	Infectious and Non-Infectious Diseases of Fur Animals, Including Rabbits			
ADP a6-5	Enteric Disease Complex of Rabbits	Fontana, Calif.	Yes	6-A
ADP a6-6	Respiratory Disease Complex of Rabbits	Fontana, Calif.	No	
ADP a6-7	Field and Laboratory Studies of Diseases of Fur Animals	Pullman, Washington	Yes	6-B
ADP a6-8	Studies on the persistence and transmission of viral and rickettsial diseases in helminths associated with diseases of fur animals	Pullman, Washington	Yes	6-C

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Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress	Line Proj. Incl. in Area & Sub-Subheading
ADP a7	Miscellaneous Infectious and Non-infectious Diseases of Animals			
ADP a7-5(R)	Reservoirs, transmission and immuno logical studies of vesicular stomatitis	Ames, Iowa	No	
ADP a7-7(R)	Investigation of livestock poisoning by plants, their toxicity for different classes of livestock, and methods of treatment and prevention	Logan, Utah	Yes	7-H
		Sao Paulo, Brazil	No	
ADP a7-8(R)	Investigation of the toxicity of herbicides and herbicide-treated plants to livestock	Logan, Utah	No	
ADP a7-12(R)	Use of radioactive isotopes in studying insecticide toxicology in animals	Kerrville, Texas	Yes	7-D
ADP a7-14(R)	Fractionation, purification and characterization of the components of normal and immune sera of animals	Ames, Iowa	Yes	7-A
ADP a7-15	Investigations of bloat in ruminants	Ames, Iowa	No	
		Davis, Calif.	Yes	7-B
		College Park, Md.	Yes	7-B
		State College, Miss.	Yes	7-B
		Ithaca, New York	Yes	7-B
		Madison, Wis.	Yes	7-B
ADP a7-16	Preparedness for Laboratory Assistance in Diagnosis of Foreign Animal Diseases	Greenport, L. I. New York	Yes	7-C
ADP a7-17	Studies to develop alleviators and diagnostic tests for plant poisoning and methods to avoid harmful residues in animal tissues from ingesting chemically treated plants	Logan, Utah	Yes	7-I
ADP a7-18	Investigations in cattle and sheep of the biochemical effects of agricultural chemicals and control substances	Kerrville, Texas	Yes	7-E
		Nacogdoches, Texas	Yes	7-E
ADP a7-19	Investigations of detoxication mechanisms in cattle and sheep	Kerrville, Texas	Yes	7-F
ADP a7-20	Characterization of cytological responses to toxic actions of antiparasitic and other agricultural chemicals in cattle and sheep tissues	Kerrville, Texas	Yes	7-G
ADP a7-21	Susceptibility of wild animals to foot-and-mouth disease	Greenport, L. I. New York	Yes	7-J
ADP a7-22	Studies of the incidence and pathology of cancer and other tumors in food-producing animals	Ames, Iowa	No	
		Ankara, Turkey	No	
ADP a7-23	Toxicological and pathological effects of insecticides, herbicides, fungicides, and other agricultural chemicals on livestock and poultry	Kerrville, Texas	Yes	7-D
ADP a7-24	Mycotic Diseases of Domestic Animals	Ames, Iowa	Yes	7-K
ADP a7-25*	Investigations of the Genus Pasteurella	Ames, Iowa	No	
	*Initiated during reporting year.			

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Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress	Line Proj. Incl. in Area & Sub-Subheading
ADP a8	Foot-and-Mouth and Other Exotic Diseases of Cattle			
ADP a8-1(R)	Histopathological investigations of foot-and-mouth and other exotic infectious diseases of cattle	Greenport, L.I. New York	Yes	8-A
ADP a8-2(R)	Development of fluorescent antibody technique to locate viruses of foot-and mouth disease and other exotic diseases in tissue cells	"	Yes	8-B
ADP a8-8(R)	Immunological investigations--vaccine studies on foot-and-mouth disease	"	Yes	8-C
ADP a8-10(R)	Immunological investigations to determine the mechanisms of antibody formation using viruses of exotic animal diseases	"	No	
ADP a8-11(R)	Immune response to various types and sub-types of foot-and-mouth disease virus	"	Yes	8-D
ADP a8-12(R)	Cytological Investigations--Development of methods for production of large quantities of FMDV by tissue culture methods	"	Yes	8-E
ADP a8-13(R)	Cytological Investigations--Microcinematographic study of cellular reactions to the agents of exotic diseases	"	Yes	8-F
ADP a8-14(R)	Cytological Investigations--Establishment and characterization of cell lines and cell strains for the propagation of foot-and-mouth and other exotic disease agents of cattle	"	Yes	8-G
ADP a8-17(R)	Mechanism of the interaction between foot-and-mouth disease virus molecules and host cells	"	Yes	8-H
ADP a8-18(R)	Investigation of the genetic biochemistry of FMDV	"	Yes	8-I
ADP a8-19(R)	Effects of certain chemical and physical environments on FMDV	"	Yes	8-J
ADP a8-20(R)	Microbiological Investigations--Bulk freeze-drying of foot-and-mouth disease virus, vaccines, and antiserums	"	Yes	8-K
ADP a8-23	Investigations of Rinderpest in cattle	"	No	

Continued on next page

Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress	Line Proj. Incl. in Area & Sub-Subheading
ADP a8-24	Survival and transmission of foot-and mouth disease virus in the semen of susceptible species of animals	Greenport, L. I. New York	Yes	8-L
ADP a8 25	Identification, Purification, characterization of FMDV	"	Yes	8-M
ADP a8-26	Immuno-chemical investigations of foot-and-mouth disease	"	Yes	8-N
ADP a8-27*	Microbiological Investigations-Attenuation of Representative types of FMDV	"	No	
ADP a8-28	Survival and Inactivation of FMDV in meat and meat by-products	"	Yes	8-O
ADP a8-29	Cytological Investigations - Studies on the biological mechanisms of natural resistance and susceptibility to foot-and-mouth disease virus	"	Yes	8-P
ADP a8-30*	Biological alterations of FMDV from continued residence in cell cultures	"	No	
	Studies on Foot-and-Mouth Disease Virus	Sao Paulo, Brazil	Yes	8-Q
	* Initiated during reporting period			

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Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress	Line Proj. Incl. in Area & Sub-Subheading
ADP a9	Foot-and-Mouth and Other Exotic Diseases of Swine			
ADP a9-1(R)	Immunological investigations of foot-and-mouth disease of swine	Greenport, L. I. New York	No	
ADP a9-2(R)	Investigations of African swine fever	Kenya, East Africa Madrid, Spain	Yes Yes	9-A 9-A
ADP a9-3*	Rinderpest in Pigs	Greenport, L.I. New York	Yes	9-B
	*Initiated during reporting period			

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Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress	Line Proj. Incl. in Area & Sub-Subheading
ADP all	Foot-and-Mouth and Other Exotic Diseases of Sheep			
ADP all-1*	Immunological Investigations of Foot- and Mouth Disease of Sheep	Greenport, L.I. New York	Yes	10-A
ADP all-2*	Rinderpest in Sheep	"	Yes	10-B
	*Initiated during reporting period			

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Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress	Line Proj. Incl. in Area & Sub-Subheading
ADP bl	Parasites and Parasitic Diseases of Cattle			
ADP bl-6(R)	The ecological factors influencing gastro-intestinal nematodes of cattle	Auburn, Ala Experiment, Ga.	Yes Yes	11-A 11-A
ADP bl-12(R)	Effect of Pasture Mixtures and Pasture Management on Control of Internal Parasites	Auburn, Ala. Experiment, Ga.	No No	
ADP bl-19(R)	Influence of Diet and Nutrition of Cattle on Roundworms	Beltsville, Md.	No	
ADP bl-22	Artificial Propagation of Protozoan Parasites	Beltsville, Md.	Yes	11-B
ADP bl-23(R)	Host-Parasite Relationship of Coccidia of Cattle	Auburn, Ala.	No	
ADP bl-24	Ecology and Immunology of the Cattle Lungworm	Beltsville, Md.	Yes	11-C
ADP bl-25	Clinical and Physiological Aspects of Roundworm Parasitism in Cattle, including Anthelmintic Treatment	Davis, Calif.	Yes	11-D
ADP bl-26	Investigations of Trichomonad Parasites	Logan, Utah Logan, Utah	Yes Yes	11-E 11-E
ADP bl-27	Host-Parasite Relationships of Intestinal Worms, Cooperia, spp. in Cattle	Auburn, Ala.	Yes	11-F
ADP bl-28	Factors Influencing Internal Parasitism of Grazing Cattle	Beltsville, Md.	Yes	11-G
ADP bl-29	Etiology and Immune Response of Cattle to Winter Coccidiosis	Logan, Utah Bozeman, Mont.	Yes Yes	11-H 11-H
ADP bl-30	Investigations of Anaplasmosis of Cattle	Beltsville, Md. Kerrville, Texas Montevideo, Uruguay	Yes Yes Yes	11-I 11-I 11-I
ADP bl-31	The Interrelationship of Diet and Parasitic Infection in the Production of Cattle	Auburn, Ala.	No	
ADP bl-32	The Histochemistry of Gastro-intestinal Nematodes of Cattle	Auburn, Ala.	No	
ADP bl-33*	Worm Parasites of Cattle--Emphasis on Stephanofilarial Species	University Park, New Mexico	Yes	11-J
	*Initiated during reporting period			

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Work & Line Project Nu Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress	Line Proj. Incl. in Area & Sub-Subheading
ADP b2	Parasites and Parasitic Diseases of Swine			
ADP b2-4(R)	The effect of anthelmintic treatment on rate of gain when administered to parasitized pigs of different ages and on different nutrition levels	Tifton, Georgia	No	
ADP b2-10(R)	Investigation of the bionomics and pathogenicity of the swine whipworm	Beltsville, Md.	No	
ADP b2-11(R)	Control of swine kidney worms by herd management	Beltsville, Md.	Yes	12-A
ADP b2-12(R)	Investigations of the swine intestinal roundworm, Ascaris suum.	Lincoln, Nebraska	Yes	12-C
ADP b2-15	Investigations of strains of Trichinella spiralis resistant to heat and cold and modes of transmission of the parasite	Beltsville, Md.	No	
ADP b2-17*	Studies of Strongyloides ransomi infections in baby pigs	Tifton, Georgia	Yes	12-B
ADP b2-18*	Evaluation of biochemical and other aspects of the host-parasite relationship in the development and severity of helminthiases of swine	Beltsville, Md.	No	

*Initiated during reporting period

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Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress	Line Proj. Incl. in Area & Sub-Subheading
ADP b3	Parasites and Parasitic Diseases of Sheep and Goats			
ADP b3-7(R)	Effect of intestinal roundworms on the exogenous and endogenous utilization of protein, carbohydrate, and various minerals by sheep	Fargo, N. Dakota	Yes	13-E
ADP b3-13	Investigations of parasitism of sheep in the South	Auburn, Alabama	Yes	13-C
ADP b3-14	Investigations of the bionomics of coccidial parasites of sheep and goats	Beltsville, Md.	No	
ADP b3-15	Investigations on the effects of helminthic infections on serum proteins of sheep and goats	Beltsville, Md.	Yes	13-A
ADP b3-16	Investigations of gastrointestinal nematodes and nematodiases of sheep and goats and measures for their control	Beltsville, Md. Lexington, Ky.	Yes Yes	13-B 13-B
ADP b3-17	The biology of the liver fluke, Fasciola hepatica, of sheep and cattle, etc.	Bozeman, Montana	No	
ADP b3-18	The life histories, biology, pathogenesis and control of several helminth parasites of sheep occurring in the Southwest	University Park, New Mexico	Yes	13-D
ADP b3-19	Studies on the life cycles of Eimeria ahasta and Eimeria crandallis, pathogenic coccidia of sheep	Auburn, Alabama	Yes	13-C

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Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress	Line Proj. Incl. in Area & Sub-Subheading
ADP b4	Parasites and Parasitic Diseases of Poultry			
ADP b4-6*	The Bionomics of Intestinal Protozoan Parasites of Poultry	Beltsville, Md.	No	
ADP b4-8*	Investigations of the Immunology of Protozoan Parasitic Diseases of Poultry with Special Reference to Blackhead	Beltsville, Md.	No	
ADP b4-9	Investigations for Controlling Coccidiosis of Poultry	Beltsville, Md.	Yes	14-A
ADP b4-10	The Biology of the Nematode Parasite of Poultry and related birds with Special Reference to the Application of Findings to Control Measures	Beltsville, Md.	No	
ADP b4-11**	Biological Investigations of Protozoan Parasites and Parasitic Diseases of Poultry, with Special Reference to those of the Gastrointestinal Tract	Beltsville, Md.	Yes	14-B

*Discontinued during reporting year
**Initiated during reporting year

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Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress	Line Proj. Incl. in Area & Sub-Subheading
ADP b5	Treatments for Removal or Control of Parasites of Domestic Animals			
ADP b5-5(R)	Evaluation, development, and standardization of chemical methods of established or reported value for the control of parasitic diseases of livestock and poultry	Beltsville, Md.	Yes	15-A
ADP b5-6(R)	Develop new and improved anthelmintics for farm animals	Beltsville, Md. Auburn, Alabama	No Yes	 15-A
ADP b5-7(R)	Investigations of chemical prevention of parasitism in livestock	Beltsville, Md.	No	
ADP b5-9(R)	Investigations for Treatments for bovine venereal trichomoniasis	Beltsville, Md.	Yes	15-B
ADP b5-12	Investigations of parasitic and related skin diseases of cattle, sheep, and swine, with primary emphasis on chemical control and basic biology of mange and scabies	Albuquerque, New Mexico	Yes	15-C
ADP b5-13	Pathobiology of parasitic infections with special reference to the injuriousness of arthropod parasites, and the economic gain and efficiency of control measures	Albuquerque, New Mexico	Yes	15-D
ADP b5-14	Development of new methods for the control and eradication of ticks of domestic animals, with special reference to the cattle fever ticks, Boophilus annulatus and B. microplus, the principal vectors of bovine piroplasmosis	Albuquerque, New Mexico	No	
ADP b5-15	Development of new approaches and methods for the control and eradication of scabies in sheep and cattle	Albuquerque, New Mexico	No	
ADP b5-16	Control of internal parasites of livestock by management practices that will not create consumer residue hazards	Auburn, Alabama	No	
ADP b5-17	Investigations of antiparasitic agents and measures for the control of parasites belonging to the family Oestridae	Albuquerque, New Mexico	Yes	15-C

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Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress	Line Proj. Incl. in Area & Sub-Subheading
ADP b6	Miscellaneous Parasites and Parasitic Diseases			
ADP b6-2(R)	Identification of parasites of importance in regulatory and other work	Beltsville, Md.	No	
ADP b6-5(R)	Maintenance of author, subject, and host catalogues, etc.	Beltsville, Md.	Yes	16-A
ADP b6-6(R)	Maintenance of Parasite Collections	"	No	
ADP b6-9	Publication of author, subject (parasite) and host index-catalogues of medical and veterinary zoology	"	No	
ADP b6-10	Investigation of immunologic and other biologic approaches to the prevention and control of parasitic diseases	"	Yes	16-B
ADP b6-11	Studies of the chemical and physical elements of parasites and parasite-host relationships in animals	"	Yes	B6-B-1
ADP bb-12*	Taxonomic Investigations of Helminths and Other Parasites	"	No	
	Control of the liver fluke, Fasciola hepatica in domestic ruminants (E21-ADP-1)	Pulawy, Poland	Yes	16-B
	The Transmission, distribution, and bio-economics of Fascioliasis (Liver Fluke Disease) of Domestic Animals in Turkey (A22-ADP-1)	Ankara, Turkey	Yes	16-B
	Investigations on the pathogenesis of lesions produced by the local leech, Limmatis nilotica. (A10-ADP-5)	Jerusalem, Israel	No	
	*Initiated during reporting year			